THE JOURNAL

OF

GENERAL PHYSIOLOGY

EDITORS

JACQUES LOEB W. J. V. OSTERHOUT

VOLUME II, NO. 4 MARCH 20, 1920

PUBLISHED BIMONTHLY

AT MOUNT ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.

BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

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Entered as second-class matter November 25, 1918, at the Post Office at Baltimore, Md., under the Act of March, 3, 1879

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CORRECTION.

On page 209, Vol. ii, No. 3, January 20, 1920, lines 33 and 36, and page 210, line 20, for $(NH_4)_2S$ read NH_4SH.

In view of the increased cost of printing, contributors are requested to make their text as concise as possible. Tabular matter and charts are particularly costly so that these should be reduced as much as possible, and charts substituted for tabular matter whenever practicable. Charts should be drawn in India ink, on paper with a smooth surface, and large enough to stand a reduction of one-half to one-third of their original size.

A DEVICE FOR REGULATING THE TEMPERATURE OF INCUBATORS EITHER ABOVE OR BELOW ROOM TEMPERATURE.

By JOHN H. NORTHROP.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, January 23, 1920.)

It is frequently desirable in studying the effect of temperature on living organisms to have a convenient means of regulating the temperature of an incubator or series of incubators within the range of from 5–40°C. For this purpose it is essential to have a regulating device which is reliable over long periods of time with a variation of 0.2–0.3°. As far as the author is aware no convenient method has been described for this purpose.

The device described in this paper has been in use continually for several years and has proved reliable and accurate. It consists essentially in regulating the flow of water through the jacket of a double-walled incubator. This is accomplished, as shown in Fig. 1, by causing a relay to direct a stream of water either through the incubator or to waste as required by the temperature changes. This is brought about by means of a wire D soldered to the armature of the relay and attached at the other end to a glass pipette at the end of a vertical rubber tube. The current necessary to move this wire and pipette is so small that it is unnecessary to use a secondary circuit. The relay is actuated directly by the same circuit which goes through the regulator. With a relay of 150 to 200 ohms resistance, a potential difference of about 1 volt is necessary. This may conveniently be obtained from the ordinary lighting circuit by the use of lamps as shown in Fig. 1. The regulator may be any convenient type, either mercury-toluene or bi-metallic. It is placed in the regular position in the incubator.

The adjustment for temperatures higher than that of the room is as follows: Hot water is allowed to run in a slow stream from the

pipette A which is so adjusted that, when the circuit closes and the armature of the relay is pulled over, the water flows into a funnel B

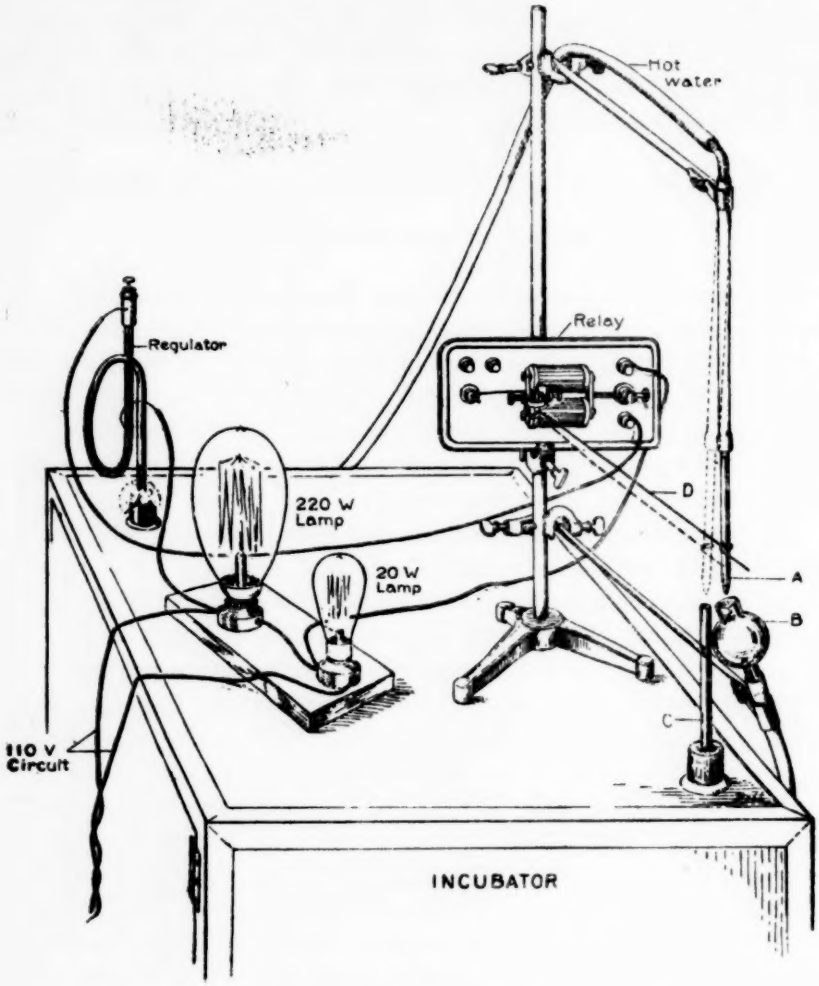

FIG. 1. Device for regulating the temperature of incubators.

placed near the opening C in the incubator jacket and thence to waste. When the circuit is broken the water flows into the incubator and so raises its temperature. The overflow from the incubator

runs off from the top of the water gauge on the side of the incubator (not shown in Fig. 1).

The adjustment for temperatures below room temperature is identical except that cold water is run through A and the relay so adjusted that the water runs into the incubator when the circuit is closed and to waste when the circuit is open. Temperatures to 8 or 10°C. may easily be maintained by the use of ice water.

When temperatures within the range of variation of the room are desired, a slow, continuous stream of cold water is run through the incubator and warm water is run through the pipette connected with the regulating device.

CONCERNING THE HEREDITARY ADAPTATION OF ORGANISMS TO HIGHER TEMPERATURE.

By JOHN H. NORTHROP.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, January 26, 1920.)

Since the time of Lamarck the theory of the adaptation of organisms to their environment and the inheritance of these adaptations has been a hotly debated question. The original theory considered that the organism responded to changes in environment in such a way as to become better fitted to it, and that these changes then became hereditary. In this form the theory is probably no longer accepted by the majority of students. There is also little doubt that structural adaptations of the individual organisms are not inheritable. A general discussion of this question is out of place here, and the reader is referred to Loeb[1] and Conklin.[2] It may be pointed out, however, that according to the accepted theory of Weismann in regard to the continuity of the germ plasm, it seems *a priori* improbable that any change in the individual could affect succeeding generations.

Morgan and his coworkers[3] have shown that structural changes are continually arising in many forms, and that these changes or mutations are inherited according to Mendel's law. There seems no reason to suppose that physiological changes might not arise in the same way. If these physiological changes were such that the organism became better fitted to a new environment, we might expect to find adaptation under some conditions, although it would not be the direct result of the changed environment. Tower[4] has reported

[1] Loeb, J., The organism as a whole from a physicochemical viewpoint, New York, 1916.

[2] Conklin, E. G., Heredity and environment in the development of men, Princeton, 1915.

[3] Morgan, T. H., Sturtevant, A. H., Muller, H. J., and Bridges, C. B. The mechanism of Mendelian heredity, New York, 1915.

[4] Tower, W. L., *Biol. Bull.*, 1917, xxxiii, 229.

experiments which apparently showed some such result. In the bacteria and other unicellular organisms, there is no doubt that cultures may be adapted to very marked changes both in temperature and concentration of toxic substances. This is, however, analogous to the adaptation of an individual multicellular organism, and cannot be considered hereditary in the sense in which the word is used in regard to higher organisms. The individual cells of a bacterial culture bear the same relation to each other as the somatic cells of a single multicellular organism, and are not at all analogous to successive generations of individuals of multicellular organisms.

The experiments reported in this paper were made with aseptic cultures of *Drosophila*. These are especially favorable for such a study for the following reasons. (1) If kept free from microorganisms the results of any experiments made with them become quite regular. (2) They have a very short generation time, about 7 days at 30°. (3) Loeb and Wasteneys[5] found that the individual *Drosophilæ* show the same marked adaptation to temperature as does *Fundulus*. In the case of the latter, Loeb and Wasteneys found that fish transferred suddenly from 10 to 35°C. died in the course of 1 to 2 hours, whereas fish transferred first to 27° for 2 or 3 days and then put at 35° were able to live indefinitely at this temperature. The results with *Drosophila* showed equally striking individual adaptations.[6] These experiments were partially repeated and confirmed in the course of the present work.

The relation of the rate of growth and of the duration of life of *Drosophila* to the temperature has been the subject of a previous paper from this laboratory.[7] It was found in that work that the insects developed normally up to a temperature of 32.5°C. Above this temperature the pupal stage was injured and no further development took place; but the larval and imago stages could live at a temperature several degrees higher. It was also found that increasing the temperature from 10 to 27.5°C. increased the rate of development of the larvæ and pupæ; but that between the temperatures of 27.5 and 32°C. the rate decreased again; *i.e.*, the larvæ grow more slowly at

[5] Loeb, J., and Wasteneys, H., *J. Exp. Zool.*, 1912, xii, 543.

[6] Loeb, J., and Wasteneys, H., unpublished data.

[7] Loeb, J., and Northrop, J. H., *J. Biol. Chem.*, 1917, xxxii, 103.

either 25 or 30° than they do at 27.5°. This secondary decrease in the rate at temperatures above 27.5° was compared with a similar decrease in the rate of enzyme action, and was ascribed to a similar cause; namely, injury and subsequent slowing up of the growth processes.

In all these experiments the eggs whose development was studied were produced by imagos which had been raised at temperatures of from 15–20°C. If there was any hereditary adaptation to higher temperature it would be expected that flies which had developed near the upper temperature limit would be able to produce eggs slightly

TABLE I.

Effect of Temperature at which Parent Generation Develops on Upper Temperature Limit for Development of the Succeeding Generation.

Temperature at which parent imagos were raised.	Temperature of development of succeeding (F_1) generation.	Condition of succeeding (F_1) generation after days noted.			
		2 days.	5 days.	7 days.	15 days.
°C.	°C.				
20	29	Eggs.	Larvæ.	Pupæ.	Imagos.
32	29	"	"	"	"
20	32	"	"	"	"
32	32	"	No larvæ.	No larvæ.	No larvæ.
20	33	"	Larvæ.	Pupæ.	Pupæ dead.
32	33	"	No larvæ.	No larvæ.	No larvæ.

more resistant to temperature (*i.e.* able to develop at a slightly higher temperature) than flies which had developed at a lower temperature. In order to test this assumption, cultures of imagos which had developed at 20 and 32° respectively were placed in incubators at 29, 32, and 33°. The development of the eggs produced by these imagos was then followed. The results are summarized in Table I.

It will be seen that those imagos which had developed at 20° and were then transferred to a temperature of 29 or 32° were able to produce eggs capable of developing into imagos at that temperature. The eggs produced at 33°, however, do not develop beyond the pupal stage. The imagos which had developed at 32° are unable to produce eggs capable of development into imagos at temperatures higher than 29°. The effect of raising *Drosophila* at high temperatures,

therefore, is to lower the upper temperature limit for the development of the succeeding generation and not to elevate it as would be expected if the adaptation to temperature was hereditary.

It might be objected that the results shown in Table I are not due to any difference either in the eggs or imago but merely to the fact that, in the case of the imagos raised at 20°, the eggs which are to give rise to the succeeding generation pass through the early stages of development within the female while at the lower temperature and so escape injury; while in the case of the cultures kept continuously at 32° the early stages of the eggs must necessarily be passed at this temperature and the eggs are thereby injured. If this was the case, only those eggs produced by the 20° culture immediately after being transferred to 32° should develop, and the ones produced later should fail to develop. This, however, is not so. Imagos raised at 20° and transferred to 32° can produce eggs, capable of developing at this temperature, for a week or 10 days after having been transferred from the lower temperature.

It was found that imagos, raised and kept permanently at a temperature of 30°, are unable to produce eggs capable of development at this temperature. If, however, they are removed from the 30° incubator within a week after emerging from the pupæ and placed at a temperature of about 20°C. for 24 hours or longer, they become able to produce eggs capable of development at 30°C. when replaced at this temperature. Table II is a summary of an experiment illustrating this point. It is necessary to remove the imagos from the higher temperature within a week or 10 days after they have emerged from the pupæ. If they are left longer at the higher temperature, the injury becomes permanent and they are no longer able to produce eggs capable of development at any temperature.

It is therefore not possible to raise more than one generation of *Drosophila* at a temperature of 29° or over unless the culture is removed to a lower temperature for at least 24 hours every generation.[8] A culture has been continued at 30° by this method of intermittent

[8] This fact may seem surprising since the organism is a tropical form. The explanation is probably that the temperature even in the tropics does not stay continuously higher than 28 or 30° for more than a week or 10 days.

cooling for ten generations without any noticeable change in the upper temperature limit. A second culture was kept continuously at 28° for fifteen generations. In this case also there has been no noticeable change in the temperature limit; *i.e.*, the organisms are still unable to grow for more than one generation at a continuous temperature of 29° or over.

TABLE II.

Effect of Placing Cultures, Raised at 30°, at 22°C.

Days.

1. 50 to 100 imagos from normal aseptic culture generation No. 89 (raised at 20°) placed at 30°C.
2. Many eggs laid.
4. Larvæ developing. Parent imagos removed.
8. Imagos of the new generation (F_1) placed at 22° and put back at 30° after varying time intervals as stated below.

Length of time at 30° C.	Time during which (F_1) cultures were left at 22°C.				
	0.25 hr.	4 hrs.	24 hrs.	96 hrs.	200 hrs.
	Stage of development of (F_2) generation cultures after days at 30°C.				
days					
3	Eggs, but no larvæ.	Eggs, but no larvæ.	Both eggs and larvæ.	Both eggs and larvæ.	Both eggs and larvæ.
5	Eggs, but no larvæ.	Eggs, but no larvæ.	Both eggs and larvæ.	Both eggs and larvæ.	Both eggs and larvæ.
10	Eggs, but no larvæ.	Eggs, but no larvæ.	Pupæ.	Pupæ.	Pupæ.
15	Eggs, but no larvæ.	Eggs, but no larvæ.	Normal imagos.	Normal imagos.	Normal imagos.

EXPERIMENTAL.

Temperature Control.—The cultures were kept in water jacketed incubators regulated as described in another paper. [9]

Food.—All cultures were fed on a sterilized suspension of yeast in water. The excess water was absorbed by cotton added to the flask, as described below.

Method of Transferring Cultures, Etc.—The insects were kept in 1 liter flat bottom Florence flasks having a side tube fused on as in a Pasteur flask. This side tube was closed with a rubber tube and glass plug, and the neck of the flask plugged with cotton. About 25 cc. of a thick suspension of yeast in water were

[9] Northrop, J. H., *J. Gen. Physiol.*, 1919–20, ii, 309.

added to the flask, the excess water was absorbed by the addition of absorbent cotton, and the flask sterilized. It is important to add sufficient cotton to absorb the water as otherwise the insects stick to the side of the flask and are drowned. In order to transfer the culture, the flask is connected to one containing the insects by means of the side tubes, using the same technique as in handling a Pasteur flask. The flies are then shaken from one flask to the other through the connecting side tubes, the flasks disconnected, and the connecting tubes flamed and replugged. In this way the organisms can be handled with as little danger of infection as cultures of bacteria.

SUMMARY.

1. Imagos of *Drosophila* raised at temperatures of from 12–28.5°C. when placed at any temperature from 15–32.5°C. produce eggs which develop normally at these temperatures.

2. Imagos raised at temperatures of from 29–32.5° and then kept permanently within these temperatures produce eggs which do not develop.

3. Imagos raised at from 28.5–32.5°C. and then placed at temperatures of from 12–25°C. produce eggs which develop normally.

4. Imagos raised at from 28.5–32.5°C. placed at 15–25°C. for 24 hours or longer and then put back into a temperature of from 28.5–32.5°C., produce eggs which will develop at the latter temperature.

5. There is no evidence of any hereditary adaptation to higher temperatures.

STEREOTROPISM AS A FUNCTION OF NEUROMUSCULAR ORGANIZATION.

By A. R. MOORE.

(From the Physiological Laboratory of Rutgers College, New Brunswick, N. J., and the Marine Biological Laboratory, Woods Hole, Mass.)

(Received for publication, January 15, 1920.)

When a starfish is put on its back, it rights itself and, though it has been known that this is a case of stereotropism, the details of the reaction have thus far not been accounted for. Thus it has happened that some authors have spoken of this reaction as a case of "trial and error" on the part of the animal. The experiments described in this paper show that the righting reaction of the starfish is a necessary consequence of the neuromuscular organization of the ray and that no room is left for the assumption of "trial and error."

It usually has been assumed by writers on the subject that the movements of the starfish, including those of righting, are accomplished entirely by the tube feet.[1] The writer has observed, however, that nicotinized individuals, in which all the tube feet except a few at the tip of the ray—the "feelers"—are retracted and inactive, begin righting movements, and in some cases complete the righting in course of half an hour or so. This indicates that the essential movements of righting can be accomplished by means of the nerves and muscles of the arms themselves without the use of the tube feet as locomotor organs. It therefore follows that the functioning of the *Asterias* ray in righting is based on principles similar to those which operate in the case of ophiurans. It seems probable that the principal rôle of the tube feet of *Asterias* in normal righting lies in their service as delicate sense organs, making possible quick and accurate functioning of the ray musculature. They also act as accessory locomotor organs and contribute to the speed of the somersault.

[1] Belonius, P., De Aquatibus, Paris, 1553.

The Musculature.

The muscles which determine the movement of the ray are classified by position as (*a*) circular and longitudinal muscles of the dorsal sheath, and (*b*) interskeletal longitudinal muscles of the ray floor.[2] The action of the sheath muscles may be demonstrated in the intact animal by stimulating the tip of the ray with a drop of 0.1 N acid. As a result the ray shortens, due to contraction of the longitudinal muscles, and suffers a decrease in diameter from the contraction of the circular ones. Similar results are to be obtained with strong stimulation of the tube feet of any part of the ray, thereby proving the nervous connection of the sensory cells of the tube feet with the muscles of the sheath.

The most complex action of the neuromuscular system is seen in the righting response. Here certain arms bend dorsally, others ventrally, in response to reciprocal impulses.[3] Obviously it greatly aids an analysis of such reactions if the investigator can produce them at will artificially or entirely apart from their normal causation. This is made possible in the case of the starfish ray through the use of the alkaloids, strychnine and nicotine.[4] With strychnine the ray bends dorsally, with nicotine ventrally. The parts played by the various groups of muscles may be shown by separating the dorsal sheath from the floor of the ray by cutting the two structures apart longitudinally. When put into either nicotine or strychnine the dorsal sheath shows dorsal flexure and rolls together so that the cut edges meet and overlap. This demonstrates physiologically the existence of longitudinal and circular muscles in the dorsal sheath. In similar fashion the presence of dorsoflex and ventroflex musculature in the floor of the ray may be shown, since in nicotine the floor bends ventrally, in strychnine dorsally.

There are therefore four muscle groups which play a part in the movements of the ray. (*a*) The longitudinal dorsoflexors of the sheath; (*b*) the circular muscles of the sheath, effective in twisting

[2] Bronn, H. G., Klassen und Ordungen des Tier-Reichs wissenschaftlich dargestellt in Wort und Bild, Leipsic, 1899, ii, 3A, 543.

[3] Loeb, J., Comparative physiology of the brain and comparative psychology, New York, 1900, 61. Moore, A. R., *Am. J. Physiol.*, 1910-11, xxvii, 207.

[4] Moore, A. R., *J. Gen. Physiol.*, 1919-20, ii, 201.

movements; (*c*) the longitudinal interskeletal dorsoflexors of the ray floor; (*d*) the longitudinal interskeletal ventroflexors of the ray floor.

It will be shown elsewhere that limited parts of each system are independently innervated and are capable of independent action. This permits the ray to bend either dorsally or ventrally at any point, and to twist the peripheral part on its long axis through any angle up to 180°.

Motor Nerve Elements.

It has been found that strychnine[5] and nicotine are without excitatory action on the neuromuscular system of the cœlenterate *Metridium*. It may be assumed then that the alkaloids in question, in the case of echinoderms and the higher forms, act on elements of the neurone especially developed in the more complex types of nervous system. The contraction of certain groups of muscles as the result of the action of nicotine or of strychnine consequently becomes an indicator of chemical excitation of the corresponding nervous elements. If we assume that the excitatory action of nicotine and strychnine is on some part of the motor neurone such as the sensory-motor junction, it would then follow as to the loci of the action of the alkaloids that (*a*) both substances excite the motor neurones of the dorsal sheath; (*b*) both sustances excite the motor neurones innervating the muscles of the ray floor which cause ventral flexure; (*c*) strychnine excites the motor neurones governing the dorsoflex musculature of the ray floor; they are refractory to nicotine.

Sensory Nerve Elements.

Ordinarily when an *Asterias* is laid on its back on the floor of an aquarium the rays begin their dorsal bending quite promptly, and righting is accomplished in 1 or 2 minutes. If, however, the aquarium jar has a cone-shaped floor and the inverted animal is placed so that its central disk rests on the apex of the cone, the righting is much delayed, since in this case the rays only by chance come into contact

[5] Moore, A. R., *Proc. Nat. Acad. Sc.*, 1917, iii, 598.

with the bottom surface. Or if a specimen of *Asterias* is suspended in the water by a thread tied around the central disk, so that the rays cannot touch a surface, dorsal bending of the rays does not occur at all. The animal hangs inert for an indefinite length of time with the rays in a position of partial ventral flexure (Fig. 1). Now hold a glass rod in contact with the back of the ray. The latter bends slowly a little distance dorsalward (Fig. 2). This proves that the sensory cells of the dorsal integument, when stimulated, cause contraction of the dorsoflexors of the sheath. Ordinarily this reflex plays a part in initiating the righting by pressing the entire ray against the bottom. This enables the sensitive "feelers" of the tip of the ray to touch bottom immediately, and from the numerous sensory cells of the am-

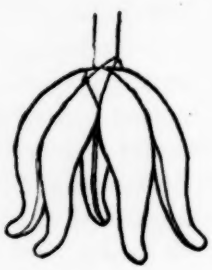

FIG. 1.

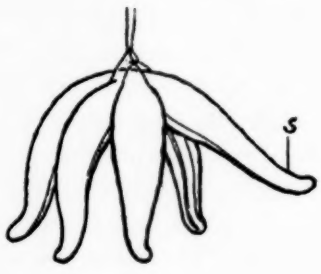

FIG. 2.

FIG. 1. Position taken by an individual *Asterias* during suspension in an aquarium. The animal is not in contact with a surface, consequently the rays are inert.

FIG. 2. A glass rod has been brought into contact with the dorsal side of the ray at the point indicated by *S*. As a result the ray has responded by bending dorsally.

bulacral disks impulses go to the musculature facilitating flexure. As the tube feet secure hold, that part of the ray straightens and actual bending occurs only centralward to the point where the last tube feet have touched bottom.

That the muscular responses of the later phases of righting are due to stimulation of the sensory cells of the tube feet may be shown in the following way. Place a medium sized *Asterias* ventral side up on a glass plate. Stimulate the sensory cells of the tube feet by touching these organs at the tip of the ray with a loop of thread dipped in 0.05 N HCl. At once the tip bends dorsally (Fig. 3). Now touch the tube

feet near the middle of the ray with the acid thread. Two results appear; (*a*) the tip returns to its former position, and (*b*) the ray bends dorsally immediately central to the point of the last application of the acid (Fig. 4). Stimulation of the sensory cells of the tube feet, therefore, results in dorsal flexure of the ray central to the point of stimulation, and inhibition distal to this point. It was found as a rule that strychnine abolished this phase of inhibition, so that in a thoroughly strychninized ray stimulation of the tube feet at any point resulted in dorsal flexure of the entire ray.

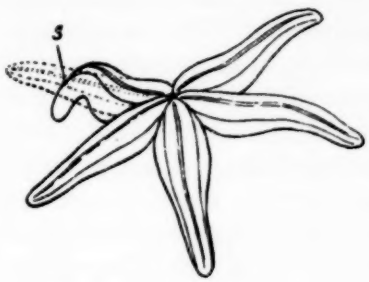

FIG. 3.

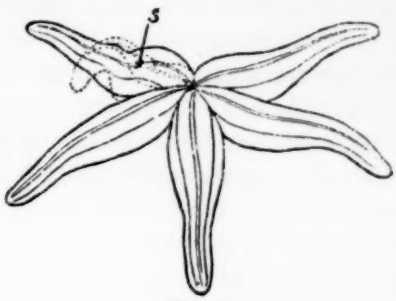

FIG. 4.

FIG. 3. The specimen lies ventral side up on a glass plate. A thread previously dipped in 0.05 N HCl has touched the extended ray (indicated by the dotted outline) near the tip as at *S*, with the result that the distal end of the ray bends sharply dorsalward.

FIG. 4. With the ray in the position of Fig. 3, shown here by dotted outline, the acid stimulus is applied to the tube feet near the center of the ray. As a result the distal half is extended to the normal position but dorsal flexure occurs central to the point of stimulation.

CONCLUSIONS.

From what has been said concerning the neuromuscular organization of the *Asterias* ray, it is possible to arrive at certain definite conclusions regarding the nature of the stereotropism of that organ. The righting movements from the start are not haphazard; *i.e.*, they cannot be explained on any hypothesis of "trial and error." The excitation of the sensory cells of the dorsal sheath initiates dorsal flexure of the ray. This movement makes it possible for the tube feet of the tip to touch bottom and start the vigorous action which follows and completes the righting If the "feelers" by chance touch bottom they initiate the reaction *de novo*.

As to the remaining phases, the stereotropic reaction of the ray is referable to the high degree of surface sensitivity of the tube feet disks and the propagation of excitatory and inhibitory impulses, resulting from stimulation, along appropriate paths to the muscles of the ray. In this respect the tube feet may be regarded as playing a rôle similar to that of tropistic receptors in general. That is, unequal stimulation of the receptors causes a corresponding inequality of tone or of contraction in the musculature which ultimately results in an equilibrium of orientation to the factor involved.

It is evident that as a mechanism for righting stereotropism differs in one important respect from other tropisms. The latter depend for their operation upon unilateral effects in organisms which are dynamically bilaterally symmetrical. In stereotropism the sensitivity of the organism is not distributed in bilaterally symmetrical fashion. As a rule only the ventral side is stereosensitive; *i.e.*, the sensitivity is unilaterally distributed. There is a tropistic action in such cases because of the fact that when the sensitive surface is only partially stimulated an unequal contraction of the musculature follows and this as a result brings the sensitive surface into such a position that it is all equally stimulated. Equal muscle tone follows and the organism is in equilibrium with its environment.[6]

[6] Jennings (Jennings, H. S., *Univ. California Pub., Zoology*, 1907–08, iv, 156) believes that he has discovered in *Asterias* a capacity for learning in connection with this reaction; that is, a ray ordinarily passive during righting could be induced by repeated suppression of the other more active rays to undertake righting movements. In describing his experiments Jennings states that the active rays were prevented from taking part in the righting by "stimulating their tube feet with a glass rod." In 1910 (Moore, A. R., *Biol. Bull.* 1910, xix, 235), I called attention to the fact that Jennings' result did not prove the use of memory or hysteresis of any kind in this functioning of a previously passive ray, because strong stimulation or a slight injury of any sort to the other rays renders them inactive and forces the use of the ray in question. By "stimulating with a glass rod," Jennings simply diminished the reacting capacity of the active rays, or, in other words, raised their threshold either to a point equal to or above that of the ray he was seeking to "teach." The experiment may be much simplified by making a single stroke with a glass rod in the ambulacral groove of each of the four active rays. Animals treated in this way have been kept under observation and found to resume the use of the treated rays only after 1 or 2 weeks. In the interval righting is always performed by the previously inactive ray which, however, had not been stroked along the groove with a glass rod.

REGENERATION AND NEOTENY.

By EDUARD UHLENHUTH.

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(Received for publication, January 29, 1920.)

According to Kamerer, it is possible to prevent metamorphosis in the caudate amphibians by amputation of the legs or tails of the larvæ. In my attempts to produce neoteny in the larvæ of salamanders, however, Kammerer's method has been tried without success, my experiments failing completely to confirm Kammerer's positive statements with regard to the effectiveness of the method.

Dumeril[1] observed that among several larvæ of *Ambystoma tigrinum*, which were the offspring of neotenous animals (axolotls), only those metamorphosed which had been deprived by their comrades of legs and part of their tails. He believed, therefore, in contradistinction to Kammerer, that regeneration may induce metamorphosis in larvæ which without regeneration would become neotenous. Later, however, he observed that larvæ of the same lot, which had not been mutilated, also metamorphosed; hence he was finally convinced that regeneration had nothing to do with metamorphosis of the larvæ.

Kammerer[2] experimented not only on the larvæ of caudate amphibians (*Triton cristatus* and *Triton alpestris*), but also on those of *Salientia*. He amputated the limbs as well as the tail. He concluded from his experiments that

"Injuries of any sort effect metamorphosis in directly opposite ways in Urodela and Anura as demonstrated with greatest certainty in the experiments. While in the salamander larvæ neoteny is brought about without the slightest difficulty, if only one limb or a piece of the tail is removed, the same procedure induces a rapid appearance of the transformation symptoms in the tadpole."[3]

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[1] Dumeril, A., *Ann. Sc. Nat. Zool.*, 1867, vii, 229.

[2] Kammerer, P., *Arch. Entwcklngsmechn. Organ.*, 1905, xix, 148.

[3] Kammerer, P., *Arch. Entwcklngsmechn. Organ.*, 1905, xix, 176.

Though such a difference between two different groups of animals is possible, it does not seem likely that metamorphosis should be controlled in different groups of amphibians by mechanisms so different; at least such an assumption does not seem justified by the evidence presented in Kammerer's paper. In the first place, that which Kammerer calls neoteny in his *Triton* larvæ is, as far as his recorded data are concerned, only a difference of 1 month. In one instance the operated animals transformed 1 month later than the controls; in the second experiment the operated larvæ had not metamorphosed 14 days after the controls had completed metamorphosis; at this time the experiments were discontinued.[4] Nowhere do we find any indication that the sex organs were actually examined to make sure that they had developed at a more rapid rate than the rest of the organism. Nor do we find any proof that these small differences had not been produced merely by differences in the quantity of food or that they were not due to the fact that the larvæ of the different sets were the offspring of different females. Since the same objections could be raised with regard to his experiments on tadpoles, it becomes doubtful not only that so fundamental a difference exists between *Caudata* and *Salientia* as that claimed by him, but also whether amputation and regeneration had any effect on the metamorphosis of Kammerer's larvæ at all. In a later article[5] he emphasized the fact that the retardation of metamorphosis in his larvæ was not due to a retardation of growth because of insufficient food; he says:

"Individuals particularly suited for the production of the phenomena of neoteny are those which have been subjected to experiments on regeneration, since they as a rule retain . . . for a long time after the removed parts have been replaced the larval condition without showing any particular inhibition of the general growth of the body; hence they turn into truly neotenous, not into starved larvæ!"[6]

It is in this case of course extremely difficult to form any opinion about the causes which lead to retarded metamorphosis, since apparently these animals were well fed, but it is well known, and we have

[4] Kammerer, P., *Arch. Entwcklngsmechn. Organ.*, 1904, xvii, Experiment XI, p. 167, and Experiment XII, p. 168.

[5] Kammerer, P., *Arch. Entwcklngsmechn. Organ.*, 1904, xvii, 165.

[6] Kammerer, P., *Arch. Entwcklngsmechn. Organ.*, 1904, xvii, 240.

discussed it in a previous article,[7] that the larvæ of all species so far examined must grow longer in low temperature than in high temperature before metamorphosis can take place, without, however, under these circumstances becoming neotenous. Unfortunately Kammerer does not mention at what temperatures his larvæ were kept. In the same article Kammerer refers to neotenous larvæ of *Salamandra maculosa*, 2 years old; it is very probable that these larvæ were truly neotenous. The experiments reported in this paper make it, however, very doubtful that this result could have been obtained by merely cutting off the limbs and the tails. I tested the effect of the removal of the limbs and of the tail followed by regeneration of these parts first in the species *Ambystoma opacum*. In the fall of 1916 the eggs of one female were collected and twenty-eight of them divided into four series; two series (E and G), consisting of six larvæ each, were used as experimental series, E being kept at approximately 25°C., G at approximately 15°C.; each experimental series was controlled by a series consisting of eight animals (A and C). The larvæ were measured and examined at least once every week. They were kept in individual jars, and individual records were made. Both fore limbs were removed from the larvæ of Series E and G 46 days after hatching, and 88 days after hatching 50 per cent of the tail was cut off. In Series E the regeneration of the legs was nearly completed 102 days after hatching, *i.e.* 80 days before metamorphosis; in Series G the legs had not regenerated to their normal length 109 days after hatching, but from this time on regeneration occurred at a very slow rate, and the animals never possessed legs of normal length. Regeneration of the tails was most vigorous during the first weeks after they had been cut off but continued in both series throughout the larval period.

Table I shows the result of this experiment. For reasons discussed in other papers,[7] metamorphosis was regarded as taking place at the time when the first molt occurred; consequently the figures recorded in this table represent the number of days after which the animals shed their skins for the first time. In several larvæ this figure was not recorded, the date when they were set on land being recorded instead. At 25°C. this was done on the same day as the first molt, or 1 day

[7] Uhlenhuth, E., *J. Gen. Physiol.*, 1918–19, i, 525.

later, and the error caused by this difference is very small. At 15°C. in only one instance was the day of the first molting not recorded, and the error in this instance may amount to from 1 to 4 days. As the figures show, there was no difference between the controls and the experimentals at 15°C., both series metamorphosing 243 days

TABLE I.

Regeneration and Metamorphosis in Ambystoma opacum.

All four series fed on earthworms; A and E kept at approximately 25°C. average temperature, C and G approximately 15°C. average temperature. In E and G, at 46 days after hatching, both fore limbs were cut off, and at 88 days 50 per cent of the tails was cut off.

No.	25°C.		15°C.	
	A Control.	E Regenerating.	C Control.	G Regenerating.
	days	*days*	*days*	*days*
1	191(Land.)	216(Land.)		236
2	200	206	241	235
3	169(Land.)	182(Land.)	249	+
4	184	+	234	239
5	186	209(Land.)	247	+
6	+	199	+	261(Land.)
7	172		242	
8	199		+	
Average	186	202	243	243
Difference		16		0

after hatching. At 25°C. the difference was very small; the regenerating series metamorphosed only 16 days later than the controls.

These results show that in *Ambystoma opacum* neoteny cannot be produced by removal of parts of the body and their regeneration. Beyond this no conclusions can be safely drawn. It should be mentioned, however, that in all four series approximately the same amount of food was available for the animals. The earthworms which served as food were given in pieces of approximately the same size. Since, however, the amount of food in these series was not large enough to cover the demand of normal growth, the animals were partly starved, as may be seen also from the length of the larval period, which amounts

to 186 days in Series A, while it is only 60 to 100 days at 25°C. if an unlimited supply of food is allowed the larvæ. For this reason it is possible that at high temperature (25°C.), at which regeneration proceeds at a more rapid rate than in lower temperatures, the regenerating larvæ were less well supplied with food than the controls, notwithstanding that both received an equal amount, and that this circum-

TABLE II.

Regeneration and Metamorphosis in Ambystoma tigrinum.

Both series kept at 15°C. In Series LVI the fore legs were removed at 26 days, from 4.5 to 11 cm. of the tails were removed at 47, 61, 68, 82, 96, 110, 124, and 138 days, and the hind legs were removed at 61 days.

No.	XLVIII Control.	LVI Regenerating.
	days	*days*
1	123	124
2	131	145
3	145	148
4	122	130
5	120	148
6	137	122
Average.....	130	136

stance caused the delay in metamorphosis in Series E. The experiments were apparently unsatisfactory also because they did not warrant a generalization of the conclusion that neoteny could not be brought about by removal of the parts of the body, since the species used might be less prone to neoteny than *Triton* and *Salamandra maculosa*.

Accordingly the experiment was repeated in the spring of 1919 with the larvæ of *Ambystoma tigrinum*, a species frequently found in neotenous condition. With respect to food, a more satisfactory condition was established by placing in the jars every day an amount of earthworms greater than was required by the larvæ. Two series, a regenerating (LVI) and a control (XLVIII), were kept at 15°C.; both were the offspring of the same female, and each consisted of six animals. They were kept in separate jars and the observations

recorded individually. In Series LVI the fore limbs were removed at 26 days, the hind limbs at 61 days, and 11 cm. of the tail at 47 days after hatching. To assure continuous regeneration the tails were clipped as soon as part of the previously removed tips had regenerated (at 47, 61, 68, 82, 96, 110, and 124 days after hatching).

The result is summarized in Table II. It was practically the same as that of the first experiment. The larvæ of Series LVI metamorphosed (*i.e.* shed the skin for the first time) 6 days later than the controls. Since this difference is smaller than the differences observed among the larvæ of the same series it may be said that in both series the larvæ metamorphosed at the same time. And certainly there was no neoteny produced by removal of even considerable amounts of tissue (42.4 mm. of tail were removed by the successive clippings of the whole larval period), though the species used is one which would be expected to yield readily to influences producing neoteny in a species like *Salamandra maculosa*, which only rarely is found in neotenous condition.

CONCLUSIONS.

It is apparently quite certain that removal of parts of the body (limbs, tail) followed by regeneration of these parts (1) does not produce neoteny in the larvæ of salamanders, and (2) has no influence upon metamorphosis.

COMPARATIVE STUDIES ON RESPIRATION.

X. Toxic and Antagonistic Effects of Magnesium in Relation to the Respiration of Bacillus subtilis.

By MATILDA MOLDENHAUER BROOKS.

(From the Laboratory of Plant Physiology, Harvard University, Cambridge, and the Marine Biological Laboratory, Woods Hole.)

(Received for publication, January 14, 1920.)

The behavior of magnesium is of special interest since in studies on antagonism magnesium has been found to occupy an intermediate position between the monovalent and the bivalent metals. In many cases it can act as an antagonist to calcium as well as to sodium. As no investigations on respiration have been undertaken from this point of view, the writer has made some experiments on this subject.

The experiments of Lipman[1] on the rate of ammonification of *Bacillus subtilis* have shown that there is some antagonism between sodium and magnesium. On the other hand he found no antagonism, but increased toxicity, when magnesium and calcium were combined.[2] Kelley,[3] in studying the ammonification and nitrification of certain soils, found no antagonism between magnesium and sodium. This sort of antagonism was observed, however, in the case of nitrogen fixation by *Azotobacter chroococcum*, as reported by Lipman and Burgess.[4]

In view of these facts it seemed desirable to study the effects of magnesium upon the respiration of *Bacillus subtilis*. The technique employed was the same as that described in a previous publication.[5]

[1] Lipman, C. B., *Bot. Gaz.*, 1910, xlix, 207.
[2] Lipman, C. B., *Bot. Gaz.*, 1909, xlviii, 105.
[3] Kelley, W. P., *Univ. of California Pub., Agric. Sc.*, 1912, i, 39.
[4] Lipman, C. B., and Burgess, P. S., *J. Agric. Sc.*, 1914, vi, 484.
[5] Brooks, M. M., *J. Gen. Physiol.*, 1919-20, ii, 5.

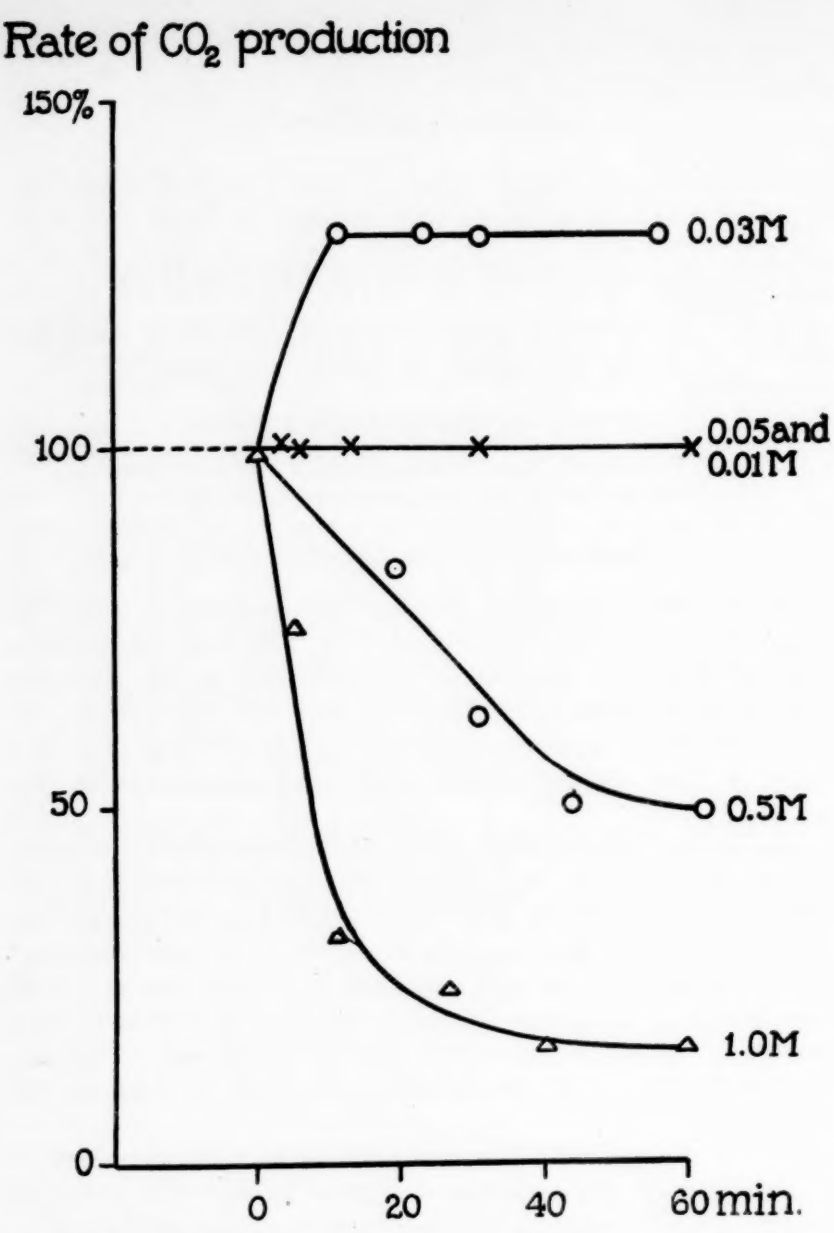

FIG. 1. Curves showing the rate of respiration of *Bacillus subtilis* (expressed as per cent of the normal) in 0.03, 0.05, 0.01, 0.5, and 1.0 M $MgCl_2$. The zero point on the abscissa denotes the beginning of exposure to the salt solution; previous to this the bacteria were in 0.75 per cent solution of dextrose in distilled water. The normal rate (which is taken as 100 per cent) represents a change in pH value from 7.78 to 7.60 in a number of seconds depending upon the amount of bacterial suspension used, usually 30 seconds. Each curve represents a single typical experiment.

Fig. 1 shows the manner in which the rate of production of CO_2 changes under the influence of $MgCl_2$, in concentrations of 0.01, 0.05, 0.03, 0.5, and 1.0 M. During the first 10 minutes the bacteria are under normal conditions and the curve (broken line) is horizontal. After this, at the point marked 0 on the abscissa, the salt is added. For example, the addition of sufficient $MgCl_2$ to make the concentration 0.03 M produces an increase in the rate which remains constant during the period of experimentation. When the concentration is 0.05 and 0.01 M the rate is normal, while in higher concentrations there is a decrease in rate. These curves are selected from a number of similar typical curves and each represents one experiment.

Fig. 2 shows the effect of various concentrations of $MgCl_2$ upon the rate of respiration expressed as per cent of the normal. The rate indicated is that produced after the bacteria had been in contact with the salt for 1 hour. The figure shows that there is an increase in the rate of production of CO_2 at 0.03 M and a decrease in rate at concentrations higher than 0.05 M.

The effect of osmotic pressure in these experiments is probably negligible, since it has been shown in a previous paper[5] that respiration is normal in a properly balanced solution obtained by mixing 1.0 M KCl and 1.0 M $CaCl_2$ in the proper proportions.

Fig. 3 shows the antagonism between the salts. Thus Curve A shows that when 9.3 parts of 0.8 M NaCl, and 0.7 parts of 0.8 M $MgCl_2$, were added to the bacteria, the rate of respiration remained normal. The other proportions did not give normal rates.

Curve B illustrates the effect of combinations of $CaCl_2$ and $MgCl_2$ upon the rate of production of CO_2. There is no normal rate with any combination of these salts at 0.8 M concentration. There is a maximum in the curve at 8 parts of 0.8 M $CaCl_2$, combined with 2 parts of 0.8 M $MgCl_2$, where the antagonism is slight. In experimenting upon lower concentrations of Ca and Mg in the same proportions, no normal rate was obtained until the concentrations were so dilute that they would of necessity, according to their separate curves, produce normal rates. It may be added that the combination of the two monovalent salts, NaCl and KCl, does not give a normal rate.

In order to find out whether the pH value of the liquid containing the bacteria was changed when the salts were added, thereby influ-

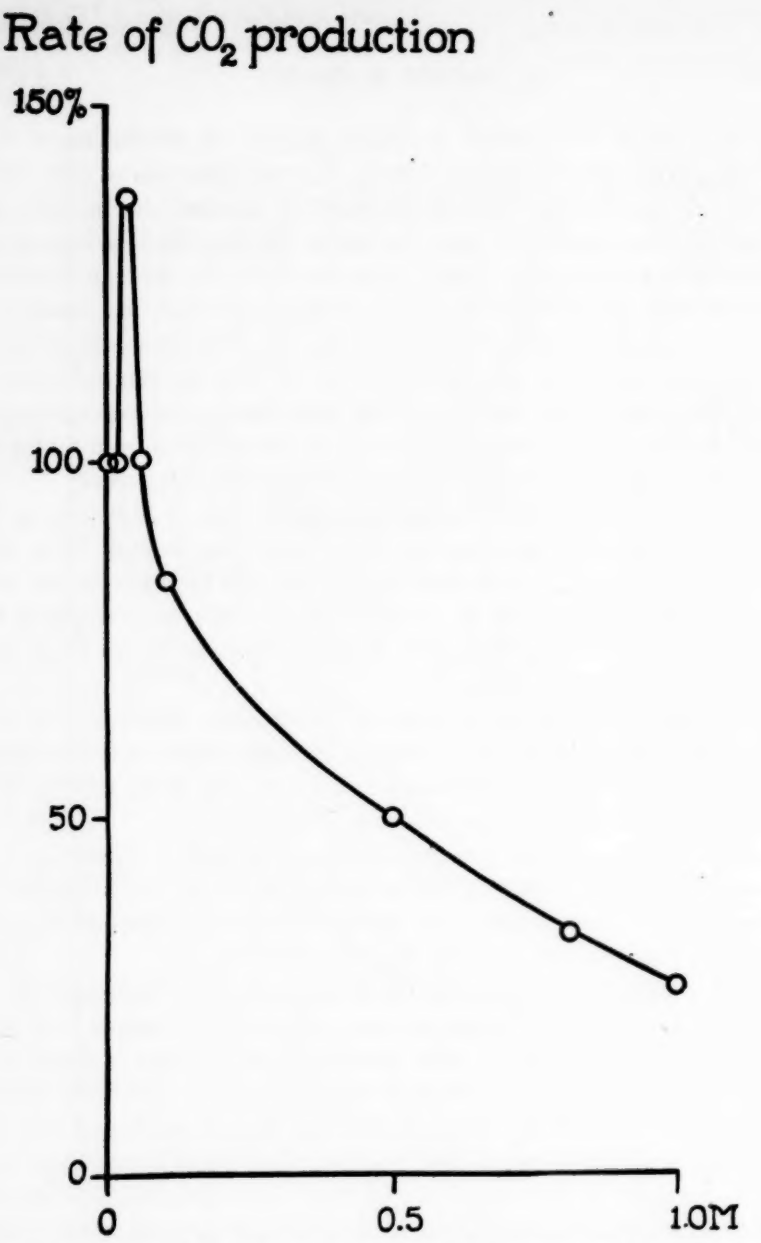

FIG. 2. Curve showing the rate of respiration of *Bacillus subtilis* (expressed as per cent of the normal) as affected by various concentrations of $MgCl_2$. The normal rate (which is taken as 100 per cent) represents a change in pH value from 7.78 to 7.60 in a number of seconds depending upon the amount of bacterial suspension used, usually 30 seconds. Average of three experiments; probable error less than 3 per cent of the mean.

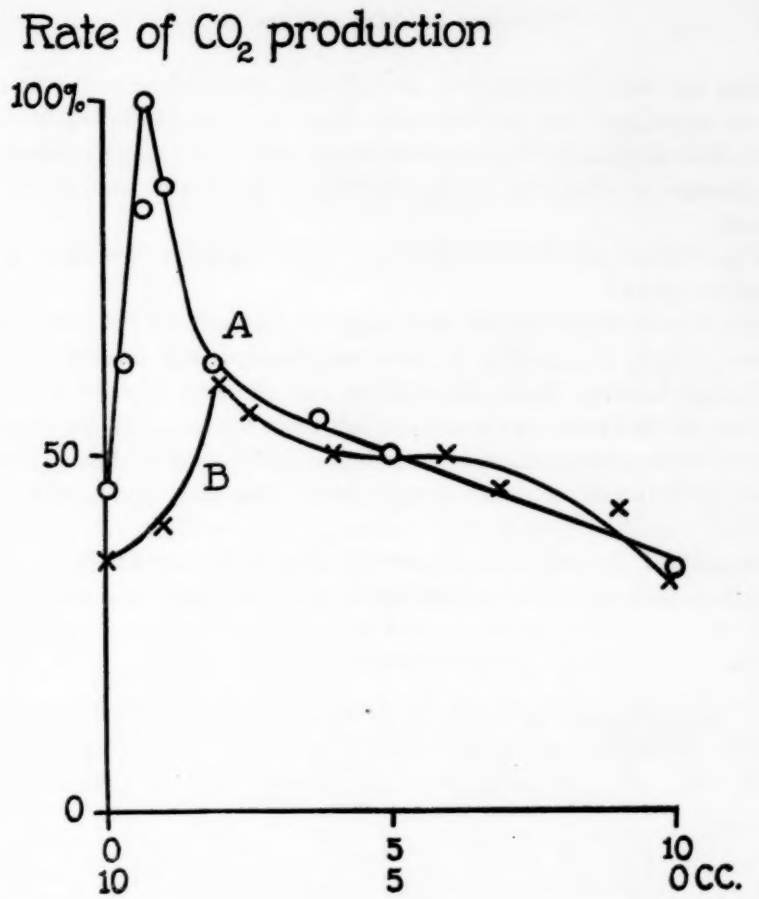

FIG. 3. Curves showing antagonism in the effect of salts on the respiration of *Bacillus subtilis* (expressed as per cent of the normal). Curve A, antagonism between 0.8 M NaCl (left), and 0.8 M $MgCl_2$ (right); Curve B, antagonism between 0.8 M $CaCl_2$ (left) and 0.8 M $MgCl_2$ (right). The ordinates represent rate of respiration (expressed as per cent of the normal); the abscissæ represent molecular proportions of the salts used. Thus, in Curve A, the ordinate at the extreme left represents the rate in 0.8 M NaCl, while the ordinate at the extreme right represents the rate in 0.8 M $MgCl_2$. The ordinate in the middle represents the rate in 50 parts of 0.8 M NaCl, and 50 parts of 0.8 M $MgCl_2$. In Curve B the ordinate at the extreme left represents the rate in 0.8 M $CaCl_2$, while the ordinate at the extreme right represents the rate in 0.8 M $MgCl_2$. The normal rate (which is taken as 100 per cent) represents a change in pH from 7.78 to 7.60 in about 30 seconds, varying according to the number of bacteria used. Curve A, average of three experiments; Curve B, average of three experiments. Probable error less than 3 per cent of the mean.

encing the rate of respiration, an indicator was added to this liquid in the apparatus; the pH value was observed to remain so nearly constant that the change in the rate of respiration could not be attributed to changes in alkalinity of the medium in which the bacteria were placed.

The control experiments gave the same results as described in a previous paper.[5]

The results are in accord with those of Lipman, except that they show a slight antagonism between magnesium and calcium. The difference between these observations and those of Lipman may be due to the differences in concentration, or in the length of the experiments, or to other matters of technique. It is possible that a difference in the strain of bacteria may have something to do with the result.

In general the results are in accord with those obtained in studies on antagonism in which criteria other than respiration are employed.

SUMMARY.

1. Concentrations of $MgCl_2$ up to 0.01 M have little effect upon the rate or respiration of *Bacillus subtilis*; at 0.03 M there is an increase in the rate, while in the higher concentrations there is a gradual decrease.

2. There is a well marked antagonism between $MgCl_2$ and NaCl, and a very slight antagonism between $MgCl_2$ and $CaCl_2$.

INTENSITY AND THE PROCESS OF PHOTORECEPTION.

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(Received for publication, January 22, 1920.)

I.

Photoreception in animals like *Mya* and *Ciona* is essentially a two-fold process. This is evidenced by the duality of the reaction time in its division into an initial exposure period and a subsequent latent period. The underlying mechanism of photoreception follows this composition of the reaction time. During the exposure or sensitization period a photochemical reaction is initiated in which a photosensitive substance S is decomposed into its precursors P and A. In the subsequent latent period these freshly formed precursors serve to catalyze an independent reaction involving the transformation of an indifferent material L into an active substance T. This active material T sets off the nervous impulse for a response (Hecht, 1918–19, *a*, *b*).

The exact interrelation between the two reactions of photoreception and the external energy is of importance in a complete understanding of the nature of the receptor mechanism. Of the two processes only the initial photochemical reaction is amenable to direct experimental modification. However, the duration of the subsequent latent period reaction is dependent on the products of the photochemical reaction. In addition, variations in the velocity of the latent period reaction are easily measureable with a stop-watch. These circumstances enable one to study quantitatively the effect of changes in the photochemical reaction on the properties of the latent period reaction.

The amount of fresh precursors produced by the initial photochemical reaction is a function of the available energy. Both components of this energy can be controlled independently, and their actions investigated. The effect of changes in the duration of exposure at con-

stant intensity has already been studied. The results show that within the investigated range of exposure the velocity of the latent period reaction is a linear function of the exposure time (Hecht, 1918–19, *b*). It is assumed that the velocity of the latent period reaction is directly proportional to the concentration of the catalytic substances *P* and *A*. Therefore the photochemical effect of the light is a linear function of its time component.

The limits between which the *time* factor may be varied in these experiments are only a few hundredths of a second apart. This is due to the short exposure which is required for a response (Hecht, 1919–20, *c*). The *intensity* factor, however, may be varied over a much greater range, and with more precision even than the time factor. The relation between the source of energy and the primary and secondary reactions may therefore be determined for a wider gradation of energy application than has been done heretofore.

With this in mind, the present series of experiments was performed. The animal used is *Mya arenaria*. For a description of the sensory properties of this animal the reader is referred to previous papers of this series (Hecht, 1919–20, *c*). The work was done at the Marine Biological Laboratory at Woods Hole, Massachusetts, during the summer of 1919.

II.

The experiments are very simple. A number of animals are thoroughly dark-adapted by being kept in a dark room for 24 hours. Using a constant source of illumination and a constant exposure time, the reaction time of each animal is determined at different distances from the light. After each observation, the animal is maintained in complete darkness for 15 minutes before the next observation is made on it. As the duration of the exposure to light is known, the latent period is found by subtracting the exposure from the reaction time. Also, since the source of illumination is a concentrated-filament, incandescent lamp, the intensities may be computed from the distances on the inverse square law. The exposure used was 0.133 second, and the source of light a 250 watt Mazda lamp. The arrangement and construction of the apparatus will be found in detail in a recent publication (Hecht, 1919–20, *c*).

Two sets of experiments were made, the first with five animals and the second with four animals. The results were identical in both series. Indeed the individual animals gave essentially the same re-

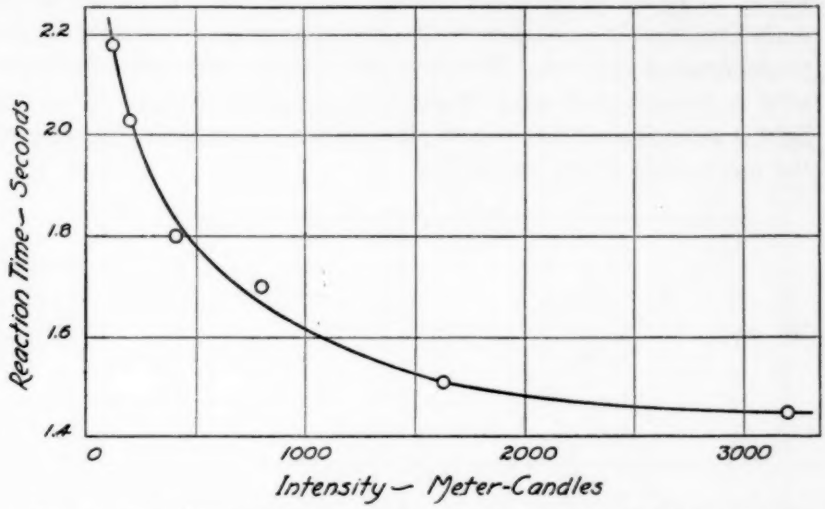

FIG. 1. Data showing the effect of variations in intensity at constant exposure on the duration of the reaction time of *Mya*. Each point is the average of fifteen readings, three each on five animals.

TABLE I.

Relation between the Latent Period and the Intensity at Constant Exposure.

Experiments 121 to 125 inclusive; exposure, 0.133 second.

Intensity.	Reaction time.	Latent period.
meter candles	*sec.*	*sec.*
118	2.18	2.05
200	2.03	1.90
408	1.80	1.67
800	1.70	1.57
1,630	1.51	1.38
3,200	1.45	1.32

sults as the averages for either set of experiments. Fig. 1 and Table I give the data for the first set of five animals. Each reaction time in the table is the average of fifteen observations, three on each of the

five animals. From Fig. 1 it is clear that the duration of the latent period varies inversely with the intensity of the stimulating light.

This conclusion is precisely what is to be expected from previous work. It indicates definitely enough that the velocity of the latent period reaction is controlled by the products of decomposition of the photochemical reaction. The data are, however, not to be dismissed with a merely qualitative treatment. A closer analysis brings to light a situation of the utmost significance for an understanding of the mechanism of photoreception.

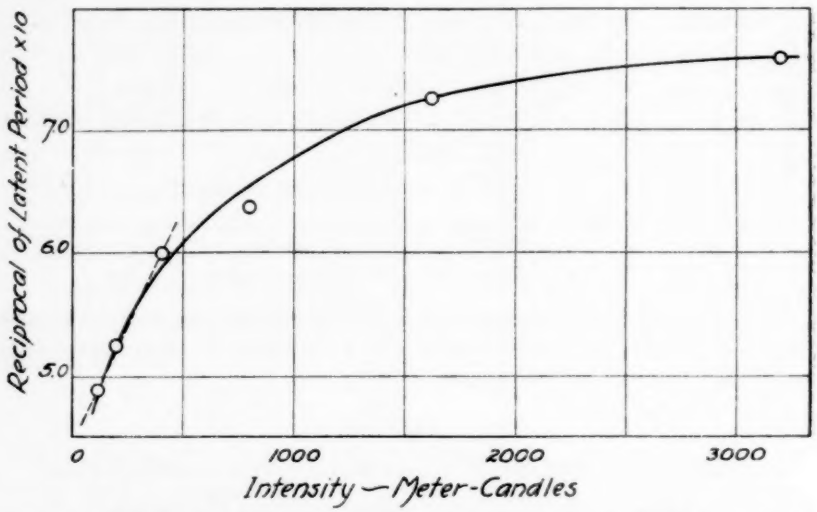

FIG. 2. Relation between the intensity at constant exposure and the velocity of the latent period reaction. The velocity as given by the ordinates is ten times the reciprocal of the latent period.

III.

The reciprocal of the latent period is a direct measure of the velocity of the reaction $L \rightarrow T$ which determines the duration of the latent period. Fig. 2 gives the connection between the intensity and the velocity of the latent period reaction. For convenience the velocity is represented as ten times the reciprocal of the latent period. This makes no difference in the theoretical deductions, because it merely changes the units in which the velocity is given. From the figure it

is at once apparent that the relation between the velocity and the intensity is not linear. What the relation is does not appear definitely, but the smoothed curve passing through the points is decidedly logarithmic in appearance. This is substantiated by Fig. 3 in which the logarithm of the intensity is used as abscissa rather than the intensity itself. The fact that the curve becomes a straight line shows that the velocity is some logarithmic function of the intensity.

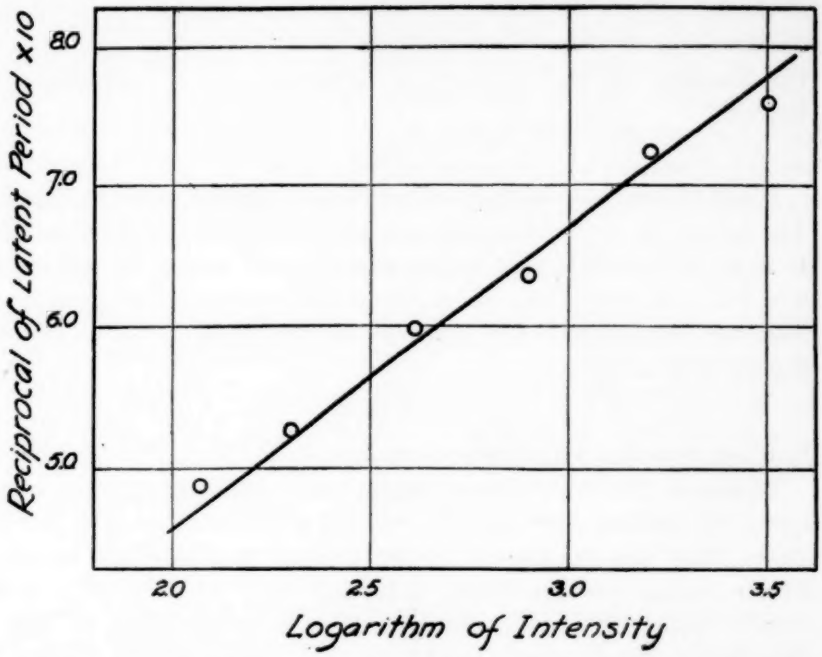

FIG. 3. Relation between the velocity of the latent period and the logarithm of the stimulating intensity. The points are the same as in Figs. 1 and 2. The straight line is drawn passing through the center of coordinates and has a slope of 2.2.

To find the exact correspondence between the two, a simple mathematical treatment is sufficient. The equation of a straight line is

$$y = a x + b \quad (1)$$

in which a is the slope of the line, x and y the abscissa and ordinate respectively, and b is the distance above the center of coordinates at

which the line crosses the y axis. The line in Fig. 3 crosses the coordinates at (0, 0). This is true graphically, and also follows from the fact that at zero intensity the velocity of the latent period reaction is zero. Therefore $b = 0$, and the equation becomes

$$y = a\,x. \quad (2)$$

Using the notation of Fig. 3, y is the velocity V, and x is the logarithm of the intensity. The numerical value of the constant a is found by dividing a given value of the ordinate by the corresponding value of the abscissa. In Fig. 3, $a = 2.2$. Equation (2) may therefore be written

$$V = 2.2 \log I. \quad (3)$$

It will be remembered that we have been using common logarithms. The factor for converting common into natural logarithms is 2.3. It is highly probable that within experimental errors, our constant $a = 2.2$ is the same thing as the factor for converting Briggsian into Naperian logarithms. The equation for the straight line in Fig. 3 should therefore be

$$V = ln\, I \quad (4)$$

in which ln means logarithms to the base e.

Equation (4) not only demonstrates the logarithmic connection between the incident light and the velocity of the latent period, but it shows that this relation is of the simplest mathematical nature. Before making any theoretical deductions from equation (4), it will clarify matters if we first consider its direct connection with the reactions which underlie photic sensitivity.

IV.

The two terms of the equation which we have just deduced represent the initial step and the final result of the double process of light sensitivity. The light decomposes a photosensitive substance into its precursors. These precursors, according to our hypothesis, then catalyze the latent period reaction, the end-product of which initiates the nervous impulse. We have discovered a simple mathematical relation between the intensity of the light and the velocity of the

latent period. The physical connection between these two is, however, not direct. It is made by the mediation of the freshly formed precursor substances. Strictly speaking then, equation (4) is subject to two interpretations. It may represent either the photochemical effect of the light or the catalytic effect of the precursors.

Actually, however, the latter interpretation is excluded. We have assumed that the relation between the concentration of precursors and the velocity of the latent period reaction is linear. "Such proportionality between concentration of the catalyst and the velocity of reaction is found to hold in numerous enzyme reactions within quite wide limits of concentration" (Euler, 1912, p. 132). Equation (4) because of its logarithmic nature cannot therefore represent the catalytic effect of the freshly formed precursors on the reaction of the latent period. It must consequently express the photochemical action of the light and should then be written

$$E = ln\ I. \quad (5)$$

Here E means photochemical effect as measured by the decomposition of the photosensitive substance S into its precursors P and A. If it were possible E would be written in grams of precursors formed by the light. As it is, it must be expressed in terms of the velocity of the latent period reaction, which is directly proportional to the concentration of precursor substances.

V.

Equation (5) as it stands is simple and clearly expresses the facts as we found them experimentally. The intensity I is the independent variable and the photochemical effect E is the dependent variable. The facts may, however, be stated in the reverse manner by saying that the intensity is an exponential function of its photochemical effect. Equation (5) then becomes

$$I = e^{E} \quad (6)$$

all the terms possessing their previous significance.

The differential of the last equation (6) states that

$$\frac{dI}{dE} = kI \quad (7)$$

which means that the increase in intensity necessary to produce an infinitely small increase in photochemical effect is directly proportioned to the intensity itself. Let the intensity I_0 produce the photochemical effect E_0, and the intensity I_1 produce the photochemical effect E_1. Then if

$$E_1 - E_0 = E \quad (8)$$

equations (6) and (7) tell us that

$$I_1 = I_0 e^E \quad (9)$$

and that

$$k = \frac{1}{E} \log \frac{I_1}{I_0} \quad (10)$$

k being a constant. In our data $k = 0.43$, which is the factor for converting natural into common logarithms used in equation (10). If natural logarithms are used, k becomes unity as we have previously found.

The significance of equations (5), (6), (7), (9), and (10), particularly of the latter two, is quite apparent. They are all different mathematical forms of the law expressing the variation of a function at a rate proportional to itself. This is a fundamental principle, which Lord Kelvin has called the "compound interest law in nature," and forms the basis of such regularities as Wilhelmy's law for the velocity of chemical reactions, and Newton's law of cooling. For our immediate interest it is significant that this very principle applies to the absorption of light passing through an absorbing medium (Lambert's law, and Beer's law).

Because of the basic similarity between the expressions for the absorption of light and for the photochemical action of light in photoreception it may possibly be that our results depend upon some constant absorbing medium in the sense organ. In that event the photochemical effect *per se* would be directly proportional to the energy transmitted by this absorbing layer to the photosensitive substance behind it.

Although a greenish black pigment is found scattered over the photosensitive siphon of *Mya* it is hardly likely that this acts as such an absorbing medium. The pigment is distributed thinly and irregu-

larly over the surface, and its maximum concentration is near the tip *within* the siphon, where the light reaches it only after it has passed through the sensitive surface. Moreover, individuals vary widely in the amount of pigment they display, some being practically free from it.

Whether our results are ultimately due to the property of an absorbing medium in the sense organ, or whether the equation represents a basic photochemical phenomenon cannot therefore be decided at present. Certain it is that some purely photochemical effects present a similar condition. For example during the period of normal exposure of a photographic plate, the photochemical effect is a logarithmic function of the intensity (Weigert, 1911, p. 86). The final meaning of our results will therefore await the elucidation of similar data in photochemistry proper.

It may be pointed out that the logarithmic relation between the intensity of the light and its effect in photoreception agrees with the general idea expressed in the Weber-Fechner law. This agreement, however, is more apparent than fundamental. The Weber-Fechner law is itself merely a psychophysiological statement of the general "compound interest" principle which our results also follow. The comparison does bring out the fact that a logarithmic relation is not peculiarly a biological phenomenon, as much of the discussion of the Weber-Fechner law may lead one to suppose. It is well known in physical chemistry, and depends on the change in a function proceeding at a rate proportional to its own magnitude.

VI.

Before concluding this paper it will be of interest to synthesize the knowledge that we have so far gained of the energy relations in photoreception. The two components of light are its intensity and the time of its action. The photochemical effect of each of these components has now been investigated, and quantitative expressions have been deduced for them. In addition we have studied the relation of these two variables to each other. Are the various findings consistent with one another, and can any additional information be gained by their combination?

We learned that for the minimum energy necessary to elicit a response the time and the intensity follow the Reciprocity Law of Bun-

sen and Roscoe (Hecht, 1919–20, *c*). This minimum photochemical effect results from the application of 5.62 meter candle seconds of energy, the intensity being inversely proportional to the time. Now that we know the individual photochemical effect of these two variables, we may determine whether their reciprocal relation obtains in the application of energy quantities greater than the minimum of 5.62 units. If the intensity is maintained constant and the time varied, the photolytic effect is directly proportional to the time (Hecht, 1918–19, *a*). This applies within an average range of approximately 50 meter candle seconds. If the Reciprocity Law holds for this range of energy values as well as for the minimum, the relation between the intensity factor and its photochemical effect should be the same, within the experimental error, as that found for the time factor. The photolytic effect of the light should thus be a linear function of its intensity factor within a range of 50 units of energy.

The broken line in Fig. 2 shows that this is true. The first three points in Fig. 2 cover a range of 408 meter candles. This gradation of intensity at an exposure of 0.133 second gives a variation of 54 units, similar to the range covered by the time factor. Within these 54 units the photochemical effect of the light is very obviously a linear function of the intensity, as the straight line in Fig. 2 shows. We may then conclude that the Reciprocity Law applies to the photoreception of *Mya* not only for the minimum energy requirement of 5.62 meter candle seconds, but for a range of 50 meter candle seconds as well.

The energy relations of the photoreceptor process therefore form a consistent scheme of things. This brings increased confidence in the results themselves as well as in their interpretation.

VII.

The investigation of the effect of intensity on the mechanism of photic sensitivity, with which this paper has been concerned, is of more than immediate interest. Most of the data on the photoreception of animals, particularly of higher vertebrates, are given in terms of intensity. Such data have heretofore been refractory to anything but a superficial treatment.

Among sensory processes in animals, the photic sensitivity of *Mya* is the only one that has so far yielded to physicochemical analysis. A broader application of the findings with *Mya* hinges to a large extent on the knowledge gained in the present investigation.

A concrete physicochemical mechanism has been proposed to account for photoreception in *Mya*. Fortunately the effect of the intensity on this mechanism has turned out to be a simple application of a general principle of physics and chemistry. We are therefore in a position to attack similar problems of perhaps wider interest to general physiology.

SUMMARY.

1. In the photosensory process of *Mya* the latent period varies inversely as the intensity of the stimulating light.

2. Quantitative analysis of the data shows that the photochemical effect of the light is a logarithmic function of its intensity, the two variables being related to each other according to the well known "compound interest" law.

3. Comparison with previous experiments demonstrates that the Reciprocity Law of Bunsen and Roscoe applies to the photosensory process not only for the minimum energy required for a response, but for a much greater range of energy application as well.

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LABYRINTH AND EQUILIBRIUM.

II. The Mechanism of the Dynamic Functions of the Labyrinth.

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(Received for publication, January 23, 1920.)

In a previous paper,[1] I have shown that the ampullæ alone, without the otoliths, suffice for all the dynamic functions of equilibrium of the ear, and that the otoliths alone, without the ampullæ, also suffice for all the dynamic functions except that of response to rotation in a horizontal plane. I wish now to point out what I believe to be the mechanisms by which these reactions are brought about.

The Dynamic Functions of the Ampullæ.

Mach, Brown, and Breuer at first attached paramount importance to the space relations of the semicircular canals. It was assumed that rotation of the head in the plane of a canal caused, by the inertia of the endolymph, a current within the canal contrary to the direction of rotation. It was supposed that the hair cells of the crista were deflected by the current and stimulation of the nerve endings resulted. Mach,[2] however, very soon saw that under the conditions existing in the labyrinth such a current could not be produced, and Breuer later admitted that the hair cells do not project into the endolymph but are covered by a gelatinous mass.

On the experimental side, Loeb[3] found that in the dogfish the canals could be cut through and even large portions could be excised without

[1] Maxwell, S. S., Labyrinth and equilibrium. I. A comparison of the effect of removal of the otolith organs and of the semicircular canals, *J. Gen. Physiol.*, 1919–20, ii, 123.

[2] Mach, E., Grundlinien der Lehre von den Bewegungsempfindungen, Leipsic, 1875.

[3] Loeb, J., Ueber Geotropismus bei Thieren, *Arch. ges. Physiol.*, 1891, xlix, 175.

affecting the compensatory movements or the functions of equilibration, and Ewald[4] stated that in the pigeon, after the canals had been ligatured, plugged, and cut, compensatory movements of the eyeballs and eye nystagmus were produced by rotation.

These experiments show that the canals are not necessary to the dynamic functions. Certain objections, however, might be raised. Loeb does not state specifically that all the canals were cut. Since I have shown that all the dynamic functions except that of response to rotation in a horizontal plane may be performed by an ear from which all the ampullæ have been removed, it would be necessary to know that the horizontal canals had been cut before the proof could be considered complete. Furthermore, in the dogfish each horizontal ampulla reacts to rotations in one direction only; this according to Ewald is not the case in the pigeon but his proof also is incomplete.

Since in the dogfish the response to horizontal rotation is brought about by the horizontal ampulla only, it would be a crucial experiment artificially to change the plane of this canal with reference to the skull of the animal and see whether this change does or does not alter the response to rotation. I have succeeded in doing this by the following method.

The right horizontal canal was laid bare for nearly the whole distance from its ampulla to the point where its posterior end reenters the vestibule. It was then ligatured and cut as far posterior as possible and the cut end was gently lifted into a vertical position, laid over against the skull, and supported there by a pledget of cotton. Its new plane was at right angles to its original plane and also at right angles to the long axis of the body. It is needless to say that in this operation extreme care must be taken not to exert the least traction on the ampulla. It is clear that with the canal in the new position rotation of the animal in a horizontal plane, that is around a dorsoventral axis, could not even theoretically give rise to a current in the canal. On rotation to the right, however, the eyes turn to the left and on rotation to the left the eyes turn to the right; that is, the ampulla whose canal is now at right angles to its normal position acts just like the

[4] Ewald, J. R., Physiologische Untersuchungen über das Endorgan des Nervus octavus, Wiesbaden, 1892.

other ampulla whose canal is still horizontal. On the other hand, rotation of the animal around its longitudinal axis (in the new plane of the canal) never produces a deviation of the eyes to the left as it might be supposed to do if the rotation causes a current in the canal and the current excites the ampulla. This experiment, then, shows conclusively that the excitation of the sensory structures in the ampulla is due to some other cause than the production of a current in the canal.

Since no further consideration need be given to the possibility of currents in the semicircular canals as the cause of the excitation which on rotation gives rise to the reflex compensatory movements we may consider other possible causes. These might be (1) effects dependent on the inertia of the mass of liquid or other material in the vestibule, or (2) due to the inertia of the contents of the individual ampullæ, or (3) to inertia effects within the sensory cells themselves. It would be impossible to decide between these *a priori*.

In my earlier experiments I found that after destruction of the structures in the vestibule I could never obtain compensatory movements on rotating the dogfish around its dorsoventral axis. For a long time I was inclined to think that the absence of the reflex was due to some sort of injury to the ampullæ, although these appeared to be as sensitive as before to direct mechanical stimulation; the slightest pressure caused decided eye movements. When, however, I was finally able to remove the otolith from the recessus utriculi by slitting open the utriculus lengthwise without tearing it across I found that the compensatory movements to rotation in the horizontal plane were not abolished.[1] Since the destruction or the transection of the utriculus abolished the reflex with no apparent reduction in the direct sensitivity of the ampulla it became clear that the utricular (and possibly the saccular) structures are essential parts of the mechanism.

In attempting to analyze more closely the arrangements of the parts concerned it is to be noticed that the movement of rotation which acts as a stimulus to any given ampulla carries foremost the side of the ampulla which bears the crista. Thus the cristæ of the anterior canals are on the lower side of their ampullæ and a rotation of the head downwards excites them; the cristæ of the posterior canals are also on their lower sides and a rotation of the head upward (back part

of the head downward) excites them. So also the crista of the right horizontal canal is on its right or outer side and the stimulus for it is rotation to the right. Of course a similar relation exists for the left ampulla. Examination of the extensive series of drawings by Retzius[5] shows that the dogfish is not a special case but that the arrangement is general.

A second fact which is significant is that the mouths of the ampullæ are continuous with the utriculus, an elongated, thin walled sac, stretched across the cavity of the vestibule and occupying only a portion, in the dogfish a not relatively large portion, of the vestibular space. Furthermore the utriculus is so attached by means of the sinus superior and other structures that a movement of the liquid contents of the vestibule might readily press it upwards towards the dorsal side of the cavity, but could have little effect to move it downwards. The relations as far as the ampulla of the anterior vertical canal is concerned are shown diagrammatically in Fig. 1. Rotation of the head downwards, that is, in the direction of the outer arrow, would tend by inertia to produce the same effect as if, with the head stationary, the perilymph was rotated in the opposite direction, as indicated by the small arrow within. This would put pressure and tension on the under side of the anterior end of the utriculus; this tension would be communicated to the ampulla and especially to its lower side which bears the crista.

In order to convince myself of the correctness or incorrectness of the above reasoning, I constructed a model by carving cavities and channels corresponding to the relations shown in Fig. 1. In these I placed a thin rubber model of the two canals shown in the figure. The canals and utriculus as well as the perilymphatic space were filled with mercury. On rotating the apparatus it could be seen that movement in one direction gave a very perceptible pull on the ampulla; movement in the opposite direction was almost without effect. It is possible that the rotation which puts the ampulla under mechanical strain would also tend to produce an increased liquid pressure within it, but this I could not determine in my present model.

[5] Retzius, G., Das Gehörorgan der Wirbelthiere. I. Das Gehörorgan der Fische und Amphibien, Stockholm, 1882.

Careful dissection shows that mechanical relations analogous to those just described hold also for the posterior ampulla and the horizontal ampulla.

I wish to point out the advantage which the vestibular mechanism possesses on account of the mass of liquid. A relatively large free mass of liquid with a relatively small surface would show more inertia effect than a small mass with a relatively large surface area.

This principle was shown in a model made by Mr. W. F. Hoyt. Rossi[6] had constructed a model of the size of a human semicircular

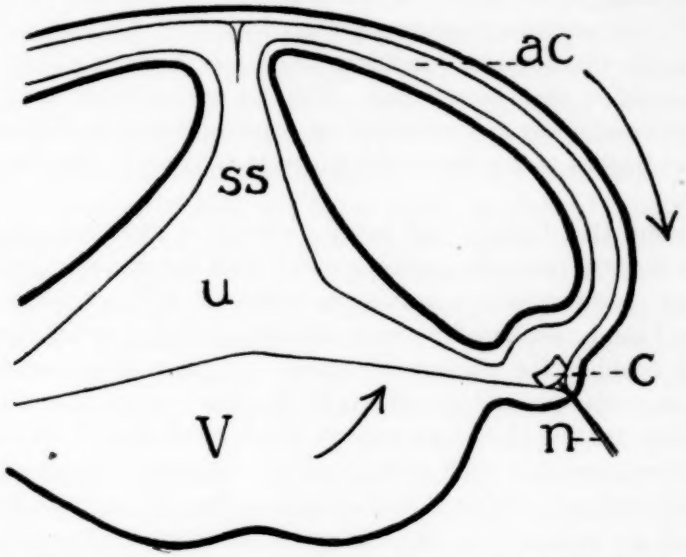

FIG. 1. Diagram to show relation of vestibular structures to ampulla; *v*, vestibule; *u*, utricle; *ss*, sinus superior; *ac*, anterior vertical canal; *c*, crista; *n*, nerve.

canal and ampulla and reported that movement of the liquid in the canal could be seen when the model was rotated. Hoyt made his model of glass for the greater transparency, and filled it with a liquid containing flakes of aluminium powder in suspension. When the model was rotated very rapidly and then suddenly stopped he could indeed see some movement in the canal, but the striking fact was that a marked movement of rotation took place in the ampulla.

[6] Rossi, G., Di un modello per studiare gli spostamenti della endolinfa nei canali semicircolari, *Arch. Fisiol.*, 1914, xii, 349.

This movement was very much more readily produced and lasted much longer than that in the canal.

It has not been a part of the problem I have set myself to find explanations for the existence of structures in the labyrinth, but, on the contrary, to find out where and how definite functions are performed. Nevertheless nearly every person to whom I have communicated these results has asked, "What then are the functions of the semicircular canals?" It may be suggested that possibly a movement of liquid does take place through the canals, not as a stimulus to the nerve endings in the cristæ, but as a means of equalization of pressure. Indeed if the ampullæ were merely diverticula from the vestibule it is conceivable that pressure conditions could arise in them which might seriously affect their functioning. It is not unreasonable to suppose that the canals provide a means for the equalization of liquid pressure quite analogous to the use of the Eustachian tubes in equalizing air pressure.

The fact that in man and many mammals a nystagmus may be caused by irrigating the auditory canal with hot or cold water and that the character of this nystagmus differs for different positions of the head can be explained perfectly without assuming an unbelievable flow of liquid in the semicircular canals. If the temperature difference can cause convection currents in the inner ear at all under the conditions existing in such an experiment, it is certainly more reasonable to suppose that such movements of convection would arise in the mass of liquid contained in the vestibule than in the much smaller space of the canals where the friction would more readily overcome the tendency to movement. These considerations would not in the least invalidate the diagnostic use which Bárány has made of the phenomenon, but they do supply a rational explanation of its causation.

The Dynamic Functions of the Otolith Organ.

Breuer and others had suggested that the otoliths are concerned only with the static functions of the ear. Importance was attached to the space relations of the different otolith masses. But Parker[7]

[7] Parker, G. H., Influence of the eyes, ears, and other allied sense organs on the movements of the dogfish, *Mustelis canis* (Mitchill), *Bull. Bureau of Fisheries*, 1909, xxix, 43.

and I each found that in the dogfish the large otolith of the sacculus has nothing to do with equilibrium; and I have shown that an ear from which the otolith of the sacculus as well as of all the ampullæ has been removed retains both static and dynamic functions. In this case the only part remaining which can mediate these functions is the small otolith organ in the recessus utriculi.

The otolith of the recessus utriculi is, in the dogfish, an oval or nearly circular mass, 3 or 4 mm. in diameter, shaped like a planoconvex lens. Its convex surface rests upon the corresponding concave surface of the macula in the bottom of the recessus. I have described it as resting on the macula, but the relation of its edges to the membranous walls suggests the idea that it is in reality partially suspended. To one who is actually performing these experiments it is a remarkably striking fact that all the functions performed by the ampullæ of the vertical canals can also be performed by this one organ. In the case of the ampullæ each one has a highly specialized function, responding to rotation in a single plane. The otolith organ on the contrary responds to rotations in all planes except the horizontal.

The approximation of the surfaces of the otolith and the macula, each almost perfectly spherical, suggests a mechanical arrangement by which any alteration of the position of the head would cause a corresponding change of pressure relations between the two structures. A pressure change of this sort could act as a stimulus to excite the compensatory movement appropriate to any particular rotation. Whether there is within the area of the macula a local differentiation of function comparable to that of the different ampullæ I have been unable to determine. The small size of the area to be explored and the difficulty of exact localization of an artificial stimulus have so far prevented an answer to this question.

STUDIES IN THE DYNAMICS OF HISTOGENESIS.

I. Tension of Differential Growth as a Stimulus to Myogenesis.

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(Received for publication, January 17, 1920.)

The prevalent opinion among embryologists in regard to the origin of muscular tissue is that of self-differentiation. This is due largely to the work of Wilson (1904) on *Dentalium*, and Conklin (1896–97, 1905) on *Cynthia* (*Styela*) *partita* and *Crepidula* in regard to the organ-forming elements of the cytoplasm, and to the experimental work of Harrison and Lewis (1904, 1905). Harrison ablated the spinal cord of the tadpole prior to the growth of the peripheral nerves into the limb buds. This operation eliminated any peculiar formation stimulus emanating from the nervous system. Still the differentiation of the contractile substance took place in the normal manner, as did the grouping of fibers into the individual muscles. Lewis (1910) draws the following conclusion based on Harrison's experiments, in regard to the genesis of cross-striated muscle:

> "Thus it is seen that all the constructive processes involved in the production of the specific structure and arrangement of the muscle-fibres take place independently of stimuli from the nervous system and of the functional activity of the muscles themselves. Cross-striated muscle tissue and the individual muscles are thus self-differentiating."

The fact that there is considerable muscular differentiation before nerves establish a connection with their corresponding muscles has been shown by Bardeen (1900, 1906–07), Harrison, and Carey (1918) in the pig embryo. There is also considerable smooth muscle differentiation in the descending colon of the pig before either the myenteric or Auerbach's plexus is detected.

Lewis (1910) endeavored to solve the problem at how early a period in the development of the ovum this power of self-differentiation of muscle tissue begins. He found by transplanting tissue from the lips of the blastopore in the early gastrula stage of the frog that this tissue later on showed muscular differentiation. The conclusion is drawn "that muscle tissue is already predetermined in the early gastrula."

The idea conveyed by the last statement is that muscular tissue is formed, *sui generis*, by some inherent predetermination and not by the agency of its surroundings nor due to its position in the whole. Lewis' view-point is in accord with Conklin's (1905) as seen in the following statement of the latter observer: "The potencies or prospective values of any blastomere are not primarily a function of its position, but rather of its material substances."

There are three theories regarding cellular differentiation; first, the "mosaic theory" of Roux (1881), later modified by Wilson (1904), Conklin (1905), Zeleny (1904), and Boveri; second, the "organization theory" of Whitman and more recently elaborated by Child (1915) in his studies on metabolic gradients and individuality; third, "the homogeneity theory" of Driesch (1894, 1899). Driesch considers the peculiar organizing quality of protoplasm as due to the expression of a mysterious force wholly different from any in the inorganic world.

His, Roux (1881, 1892, 1893), Wilson (1892, 1893, 1897, 1904), and Conklin (1905) lay emphasis upon the cell as the key to all ultimate biological problems. Whitman, on the other hand, points out the inadequacy of the cell theory to development. "That organization precedes cell formation and regulates it, rather than the reverse, is a conclusion that forces itself upon us from many sides," is a summary of his studies. Morgan (1895, 1898) had deduced the idea from his studies on regeneration that the multicellular individual is a whole in the same sense that the unicellular form is a whole. Child (1899, 1915) also lays emphasis on the fact that it is the "organism—the individual, which is the unit and not the cell." Differentiation of a single cell, consequently, according to Child and Whitman, is a function of its position in the whole. This view is also upheld by Driesch (1894). Wilson and Conklin, on the other hand, conclude that potencies are functions of the material substance of the cell.

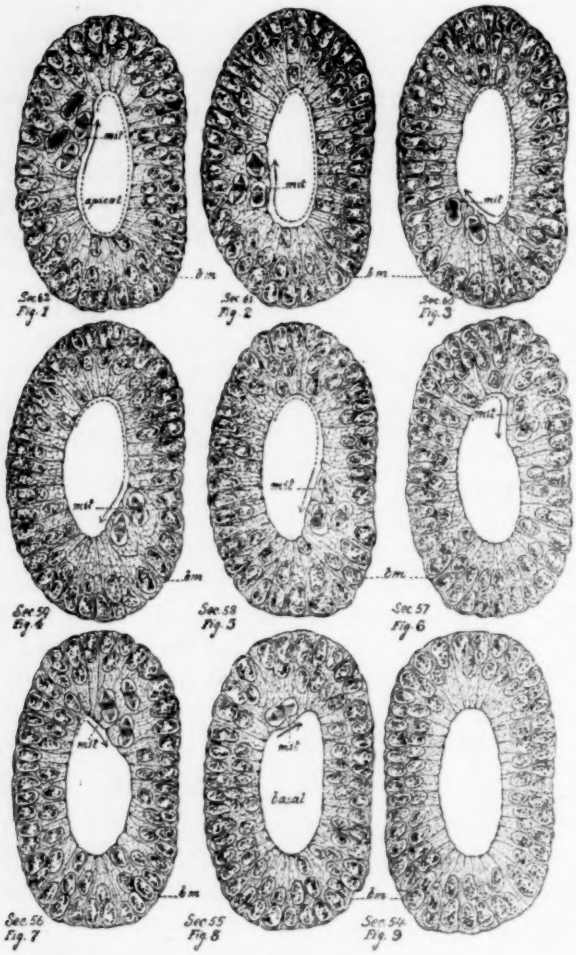
The influence of the organism as a whole in subjugating its dependent parts is convincingly shown by Loeb (1916) in his regeneration experiments on *Bryophyllum calycinum* and in his experiments on *Amblystoma* larva (1897). This influence is exerted through the blood stream by means of "hormones." The sound mechanistic attitude of Loeb toward development may be seen in the following statement: "As soon as we can show that a life phenomenon obeys a simple physical law there is no longer any need for assuming the action of non-physical agencies" (1916).

The object of this paper is not, however, to discuss historically the views of these various authors, but to emphasize certain facts in the development of muscle tissue hitherto overlooked. It is a well known fact that the embryo presents differential rates of growth. It is desired, therefore, to emphasize the fact that in embryological development there are zones of unequal or differential growth, and that the effects of these zones of growth are factors in histogenesis. The active and less active zones are defined with reference to the rate of cell division per mm. of cross-section. This principle was deduced from a series of studies on osteogenesis and myogenesis begun in 1914. Previous reports of a part of this work have been presented to the Association of American Anatomists (Carey, 1917, 1918, 1919).

It will be illuminating to search for the cellular forces outside of the immediate differentiating zone under observation. This search necessitates lower magnifications in order to enlarge our field of view. Heretofore, cytological differentiation has been studied *per se*, with magnifications of 1,000 to 2,000 diameters which considerably reduce our range of view. The higher magnifications are profitable in revealing cytological detail but the interpretation of the process is lost unless, in conjunction with the higher, intermediate magnifications are used. The employment of all magnifications of the microscope in connection with naked eye studies will reveal the interaction of related developing parts.

Early Development of the Descending Colon of the Pig.

The attention of the writer was directed to the fact, after plotting hundreds of intestinal epithelial mitotic figures, that these figures were usually confined to some definite region of the circumference of a single



FIGS. 1 to 9. Sections of the epithelial tube representing one complete turn in a dexiotropic rotation in a spiral manner of the mitotic division. The primary type is the left-handed helix. Spiral path is directed upward toward the ileocecal valve. Section 62 represents the head or apical end of the mitotic path. Sections 55 and 54 represent the tail or basal end of the mitotic spiral path. *mit*, mitosis; *bm*, basement membrane of the epithelial tube. Drawings are made with the aid of a Spencer Camera lucida. Sections 54 to 62 are from Pig Embryo 19, 24 mm. in length (Creighton Embryological Collection).

section (Figs. 1 to 9). This region was found to change at different levels of the serial sections. By graphic reconstruction this plot was found to form the path of a definite spiral. The predominant type was the left-handed helix. In one of the embryos of the twenty that were plotted this spiral was arranged as a right-handed helix (Figs. 1 to 9). The spiral itself presented a head or apical region in which mitotic figures were found to be numerous, and a tail or basal end in which there were fewer and fewer figures. The apical end of the spiral path is always directed towards the ileocecal valve and the basal end towards the rectum. Growth is, therefore, from below upwards in a spiral course. One spiral growth is quickly followed by a second which rifles a path slightly lateral to its predecessor. This in turn is followed by a third in a path still more lateral, and so on around the circumference. This intermittent rhythm of explosive spiral growth may be compared to that of the successive fire balls emitted by a roman candle in fireworks. The paths formed by this explosive spiral growth may be compared to those within the barrel of a Winchester rifle.

The most rapidly growing part of the intestine, therefore, is the epithelial tube. In embryos 10 to 25 mm. in length the descending colon grows relatively more rapidly in diameter than in length (Tables I and II). The increase in diameter is due primarily to the rapid growth of the entodermal epithelial tube and only partially to its surrounding mesenchymal cloak. The latter is relatively passive in growth with respect to the former (Fig. 10). It is during this early increase in diameter that the inner smooth muscle coat is in process of formation. The mesenchymal cells are drawn out gradually in a definite series of concentric rings. These rings appear not unlike those of the planet Saturn and the annular nebula in Lyra.

A definite centripetal force is active in the rapid spiral growth of the intestinal epithelial tube. The surrounding mesenchymal cells are thrown into an obvious series of concentric rings, according to their various densities. Those possessing the greatest density will join the outer ring in the tangential path of the force, whereas the inner ring will be composed of bodies forming a gradient of decreasing densities. The cells forming the outer ring will be most elongated.

TABLE I.

Measurements of Differential Growth of Descending Colon.

Length of embryo.	Thickness of mesenchymal wall.*	Diameter of epithelial tube.	Diameter of descending colon.	Epithelial tubular indices.†
mm.	*mm.*	*mm.*	*mm.*	*per cent*
10	0.085	0.048	0.218	6
12	0.099	0.069	0.267	8
13	0.115	0.075	0.305	8.5
14	**0.128**	0.081	0.337	10
16	0.126	0.089	0.341	14
19	0.124	0.095	0.343	18
20	0.123	0.099	0.345	20
22	0.122	0.119	0.363	25
23	0.121	0.138	0.383	30
24	0.120	0.152	0.392	38
25	0.115	0.164	0.394	40
27	0.109	0.188	0.406	44
30	0.104	0.208	0.416	46
32	0.099	0.220	0.418	47
35	0.098	0.246	0.442	48
37	0.096	0.260	0.452	50
39	0.093	0.279	0.465	52
40	0.092	0.289	0.473	54
42	0.090	0.312	0.482	57
45	0.083	0.321	0.486	61

* The mesenchymal wall begins to diminish in thickness after it reaches a width of 0.128 mm. in the 14 mm. stage of the pig embryo. This diminution is due to the tension caused by the more rapid epithelial tubular growth in diameter. Measurements made with B. and L. filar micrometer, calibrated.

† The ratio of the square of the mean diameter of the epithelial tube to that of the surrounding mesenchyme is referred to as the epithelial tubular index. It has been calculated from the following formula.

$$\left\{ \frac{\frac{(x+y)^2}{(\ 2\ )} \times 100}{\frac{(X+Y)^2}{(\ 2\ )} - \frac{(x+y)^2}{(\ 2\ )}} \right\} = Z$$

x and y are the long and short diameters of the epithelial tube respectively. X and Y are the long and short diameters of the surrounding mesenchymal tube. Z is the ratio of the epithelial tube to the mesenchymal tube.

As this concentric initial smooth muscle layer becomes differentiated it tends to restrict the diametrical growth of the epithelial tube. The epithelial mitotic figures under this restriction shift their planes of division from a right angle to a parallel position with the smooth muscle cells. This shifting results in an elongation of the intestine.

TABLE II.

Ratio of Diameter to Length of Entire Colon.

Length of embryo.	Diameter of descending colon.	Length of entire colon.	Ratio of diameter to length of entire colon.
mm.	mm.	mm.	mm.
10	0.218	1.95	1:9.9
12	0.267	2.00	1:7.5
13	0.305	2.02	1:6.6
14	0.337	2.05	**1:6.0**
16	0.341	2.10	1:6.1
19	0.343	2.20	1:6.4
20	0.345	2.30	1:6.7
22	0.363	2.75	1:7.5
23	0.383	3.00	1:7.8
24	0.392	3.50	1:8.9
25	0.394	3.95	1:10.0
27	0.406	6.00	1:14.0
30	0.416	8.00	1:19.0
32	0.418	11.00	1:26.0
35	0.442	16.95	1:36.0
37	0.452	18.00	1:39.0
39	0.465	21.00	1:45.0
40	0.473	23.00	1:48.0
42	0.482	27.00	1:56.0
45	0.486	29.00	1:59.0
Adult.	50.000	7,000.00	1:140.0

In embryos 25 to 40 mm. (Tables I and II) in length, the elongation of the descending colon is more rapid in growth than that of the diameter. It is during this period that the outer longitudinal muscular coat is in the process of formation. The rapid growth of the epithelial tube in length tends to elongate the peripheral undifferentiated mesenchymal cells which were not directly involved in the formation of the inner smooth muscular coat (Fig. 11).

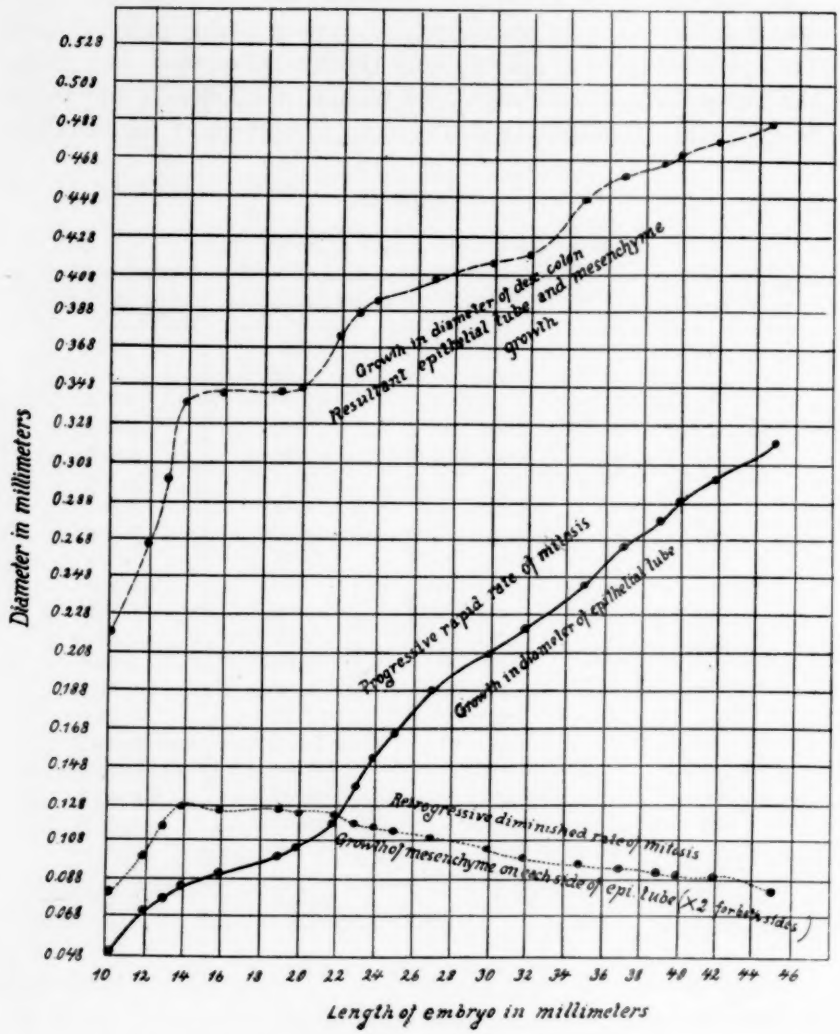


FIG. 10. Curves of differential growth of descending colon. The active growth of epithelial tube is contrasted with the passivity of the mesenchymal wall. An absolute decrease in thickness of the mesenchymal wall is seen after the stage of the 14 mm. pig embryo.

The differentiation of the outer longitudinal muscle coat therefore coincides, in time, with the rapid growth in length of the intestinal epithelium. The inner smooth muscle coat, on the other hand, is formed during the period of the rapid growth of the intestinal epithelial tube in diameter. Once the formation of the inner circular muscular rings is fairly established a resistance to growth in width is

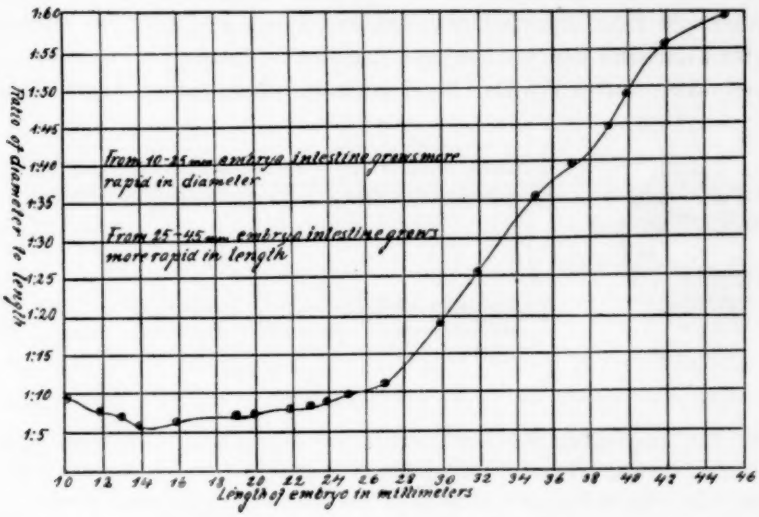


FIG. 11. Curve representing the ratio of the diameter to the length of the descending colon in embryos 10 to 45 mm. in length. Particular attention is directed to the fact that in embryos 10 to 25 mm. in length the intestine grows relatively more rapidly in diameter than in length. In embryos from 25 to 45 mm. in length the intestine grows more rapidly in length than in diameter. The inner circular smooth muscle is formed during the period of rapid growth of the intestine in diameter. During the period of the rapid growth of the intestine in length the histogenesis of the outer longitudinal smooth muscle coat is taking place.

encountered by the cells surrounding the rapidly dilating lumen. These cells then grow primarily along the path of least resistance in a longitudinal manner. At this stage the longitudinal muscle cell, spherical in shape (Fig. 12), is elongated to a spindle-shaped structure (Fig. 13).

An interesting correlation in the development of the esophagus in man may be cited. This correlation was detected in the work of

Jackson and in that of Keibel and Elze. The former investigator studied the developmental topography of the esophagus, the two latter the histogenesis of the esophagus. Jackson states that the descent of the stomach is accompanied by a great elongation of the esophagus. In a 9.4 mm. specimen, the esophagus measures 1.8 mm. At this proportion it should measure 4.3 mm. in a 22.8 mm. embryo but its actual length is found to be 8 mm. A year previous to this, Keibel and Elze reported that the esophagus in 12.5 mm. embryos shows a circular but no longitudinal muscle layer. In 17 mm. embryos, they find a circular layer with the longitudinal layer faintly

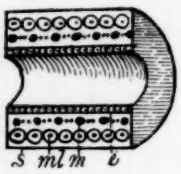


FIG. 12.

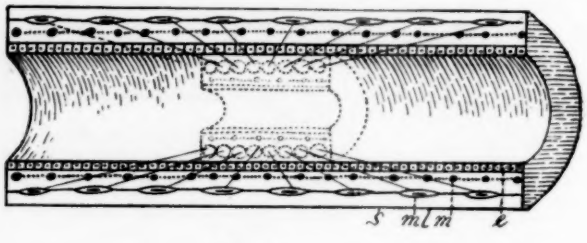


FIG. 13.

FIG. 12. Longitudinal section of intestine; *s*, peritoneal epithelium; *ml*, mesenchyme; *m*, circular muscle; *e*, epithelium.

FIG. 13. Longitudinal section of intestine schematizing the elongation of the intestine represented in Fig. 12. Due to the resistance of the inner smooth muscle layer *m*, the intestinal epithelium grows in the longitudinal path of least resistance. This results in the elongation of the outer mesenchymal cells *ml* (Fig. 12) into the elliptical or spindle cells *ml* (Fig. 13).

indicated. The histogenesis of the outer longitudinal layer of the esophagus as studied by Keibel and Elze coincides in time with the rapid elongation of the esophagus, due to the descent of the stomach, as recorded by Jackson.

Interpretation of Results.

The result of the action of a force on an elastic body is the production of a strain. If mechanical forces are at work on organic matter, they tend to produce similar results to these acting upon inert matter. Too frequently the term self-differentiation is applied to alteration of

form and internal structure of developing cells without searching the immediate environment of the specializing cells or syncytium to ascertain whether or not these changes are attributable to forces outside of the differentiating zone. This applies particularly to the differentiation of bone and muscle tissue. If a cell changes in form successively through the spherical, ellipsoid, and spindle stages it undergoes a strain. A strain is usually due to an external force which elicits internal reacting stresses in the body acted upon. Cytological differentiation is frequently a manifestation of these internal reacting stresses to forces extrinsic to the differentiating zone.

A strain is produced in certain regions of the embryo by the expansion of a rapidly dividing group of cells against a less active or relatively passive group of cells. After their differentiation the relatively passive group of cells in their turn react upon the former. This action and reaction are objectively evident by a retardation or alteration of the rate of growth, or by a change produced in the external form or internal structure of the cells involved.

In this study, the initial zone of rapid growth is found in the epithelial tube. The rapid spiral expansion of the entodermal epithelial tube reacts against the surrounding splanchnic mesenchyme with the result that the less actively growing cells of the peripheral region of the intestinal wall are elongated. Later the elongated, differentiated mesenchymal cells cause a retardation of the growth in diameter of the epithelium. Immediately following this retardation of diametrical growth the period of rapid growth in length of the intestine takes place. In this development, therefore, the influence of unequal growth zones is definitely shown as furnishing a tensional stimulus for the differentiation of muscle.

This action is diagrammatically illustrated in Figs. 14 and 15. In Fig. 15 the growth in diameter of the intestine is schematized; the rapid increase in width is shown as due primarily to the increase in the lumen. This growth is due to rapid mitotic activity of the epithelium (*e*, Fig. 14, to *e'*, Fig. 15). In the lumen of Fig. 15, the former is represented in a spiral manner, *sg*. In this growth the strain upon the surrounding mesenchymal cells *m* is illustrated. These cells are strained by the external applied forces of the progressively diverging radii. The internal reacting stresses are manifested by the changes

in shape through spherical m, ellipsoidal m', and spindle m'' cellular phases in Fig. 15. In addition to the homogeneous strain to which the cells m, m', and m'' are subjected there is a definite pressure exerted by the epithelial cells lining the expanding lumen.

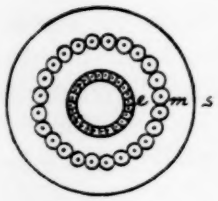


FIG. 14.

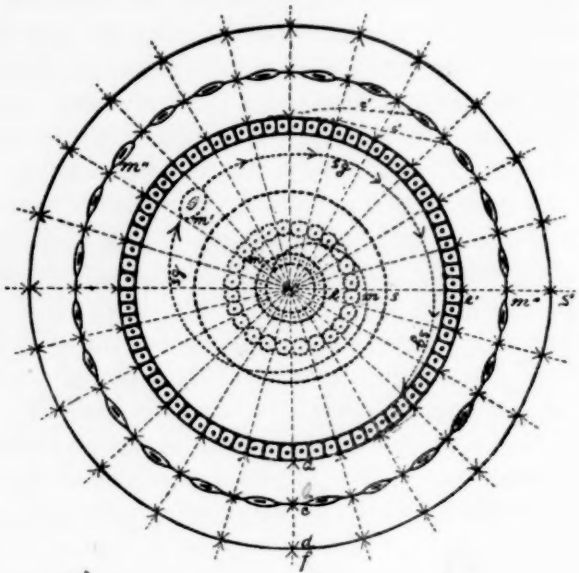


FIG. 15.

FIG. 14. Transverse section of intestine; e, epithelium; m, mesenchyme (spherical nucleus); s, peritoneal epithelium.

FIG. 15. Transverse section of intestine grown to three times the width represented in Fig. 14. Fig. 14 is represented in broken lines within the lumen. The spiral growth of the epithelium is represented by the broken lines sg. The tension, upon the mesenchyme, by the most rapidly growing epithelium, is shown in the elongated muscle cells m.'' These cells are homogeneously strained in the centrifugal path c' due to the progressively diverging radii. Cells marked m, m', and m'' represent the progressive steps in the strain ellipsoid in the differentiation of a muscle from a mesenchyme cell. The expansile force of the epithelium is shown by the double arrow a–d; the reacting resistance of the serous membrane by the line d–a. Equilibrium is established in the middle of the mesenchyme and is graphically represented by the double arrows a–b and d–c. This is another factor in the tensional elongation of the middle cells. The smooth muscle ring exerts a centrifugal reaction to the applied centripetal force of the dexiotropic spiral rotation of the epithelial tube. The mitotic figures of the descending colon primarily follow the path of a left-handed helix.

This force of expansion is represented by the arrow *a–d*. A resistance *f*, due to the peritoneal epithelium, is met. This causes a reaction *d–a*, With progressively increasing growth a zone of equilibrium of expansile and reacting forces is established in the middle, represented by the double arrows *b–a*, and *d–c*. This action and reaction of forces is another factor tending to compress the cells in the middle of the mesenchymal wall of the intestine. The action of the centripetal component of the spiral growth of epithelium in forming the rings of dense spindle-shaped muscle cells *m''* is represented by the broken curved arrows. The spiral growth of the epithelial tube in a dexiotropic rotation exerts a centripetal force upon the surrounding mesenchyme. The mesenchyme consequently exerts a simultaneous equal and opposite centrifugal force upon the epithelial tube. This growth is primarily in the form of a left-handed helix from the rectal to the ileocecal valvular regions of the large intestine. In Figs. 14 and 15 the right-handed helix is depicted.

Although by direct observation of serial sections no motion is seen, there is, however, objective evidence of homogeneous and ellipsoidal strains upon the surrounding mesenchyme. The mesenchyme is drawn out into concentric rings, the outermost of which are most viscid, by the spiral growth of the epithelial tube, roughly comparable to the increase of viscosity and concentric annular formation of egg albumin when subjected to an egg-beater.

CONCLUSION.

The genesis and maintenance of muscle tissue represents a resultant or equilibration of converging factors which are active and formative during development. *One of these factors is the tensional stresses to which the mesenchyme is subjected by a force extrinsic to the differentiating zone.* In subsequent involution or degeneration of muscular tissue during the prenatal or postnatal periods, this equilibrium is upset by altering or destroying the tensional reacting stress.

Tension is developed when a muscle contracts. Contractility is a fundamental property of protoplasm and, when manifested, tension is developed. In both, the development and specific function of muscle tissue, tensional stresses are inseparably involved. The

Ameba possesses the property of contractility in all possible directions. The function of contraction in one definite direction characterizes muscle tissue from that of undifferentiated protoplasm. What initiates the progressive series of physicochemical changes in the mesenchyme resulting in an alteration of its attribute from non-specificity to its specificity of direction of contractility? This question is answered as follows.

The mesenchyme before differentiating into muscle tissue must be subjected to a certain minimal homogeneous and ellipsoidal strain. This strain is objectively evident by an alteration of the form of the spherical nuclei, into the ellipsoidal and spindle conditions and by an elongation of the granular cytoplasm into parallel granular and continuous fibrillæ. The fibrillæ are arranged along lines of internal and reacting tensional stresses. The ends of the mesenchymal cells, in tension, must be attached to supports of which one, at least, is mobile. The tensional stresses are reactions to simultaneous forces extrinsic to the zone of myogenesis. The external forces are produced by a progressive divergence or separation of the mobile supports to which the mesenchymal cells are attached. Therefore, muscle tissue is not self-differentiating but is dependent upon an external dynamic stimulus. As regards smooth muscle this stimulus is the *tension of differential growth*.

SUMMARY.

1. The region of most active mitosis per mm. of cross-section in the intestine is the entodermal epithelial tube. The mitotic figures primarily follow the path of a right-handed helix. In one of the twenty embryos the mitotic figures describe the path of a right-handed helix.

2. The region of least active or relatively passive growth per mm. of cross-section is the mesenchyme, derived from the splanchnic mesoderm surrounding the epithelial tube.

3. The rapid expansion, due to epithelial growth in a rotating spiral manner, of the intestinal lumen is greater than the activity manifest in the surrounding mesenchyme. This causes a pressure in the latter resulting in a flattening and elongation of the mesenchymal cells. The successive changes in shape of these cells through the spherical, ellipsoidal, and spindle cellular phases are seen. The

mesenchymal wall decreases in thickness, due to tension caused by epithelial tubular dilation.

4. The rotating spiral growth of the epithelial cells causes the formation of a series of mesenchymal cellular and fibrillar concentric rings due to the centripetal force of the former.

5. The circular, smooth muscle cells are differentiated in the outer, more condensed margins of the ring. At these points the developing tensional stresses are greater than within the ring.

6. The inner circular smooth muscle coat is the first one differentiated and is incident to the rapid growth of the epithelial tube in diameter. The former soon tends to restrict the growth of the epithelial tube in diameter. The tube, pursuing the lines of least resistance, grows in length. During the period of rapid growth in length the outer longitudinal muscle coat is in the process of formation.

7. The tensional stresses to which the elongated strained mesenchymal cells are subjected appear to be a dynamic stimulus to smooth muscle differentiation.

8. From this study of a closely graded and progressive series of sections of intestinal development, the conclusion is drawn that muscle tissue is not self-differentiating, in the strict sense of the term, but that the *tension of differential growth* acts as the stimulus to smooth muscle differentiation.

The writer wishes to express his indebtedness to Professor H. von W. Schulte for his interest and his valuable suggestions; to Madame Helen Ziska for the illustrations; and to his wife for her help in reading the proof.

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THE NATURE OF THE DIRECTIVE INFLUENCE OF GRAVITY ON THE ARRANGEMENT OF ORGANS IN REGENERATION.

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(Received for publication, January 16, 1920.)

I.

In preceding papers it has been shown that gravity has a directive influence on the arrangement of certain organs in the regeneration of *Bryophyllum calycinum*. Thus, when an excised piece of stem of *Bryophyllum* is suspended horizontally in moist air, roots will grow abundantly on the lower side of the stem but not on the upper side, with the exception of the basal end where the roots will grow on both sides. In order to get abundant root formation it is necessary to preserve one of the apical leaves on such a stem since the leaf furnishes most of the material for the growth of the roots.[1]

A second case in which this directive influence shows itself is the leaf of the same plant. When we suspend an isolated leaf of *Bryophyllum* (in moist air) sidewise, in a vertical plane, roots and shoots will grow abundantly in the notches of the lower side, but less abundantly and frequently not at all in the notches of the upper side of the leaf (Fig. 1, upper row). This phenomenon was explained by the writer in the following way. Gravity causes a slightly greater collection of sap on the lower side of the organs mentioned and this causes the dormant buds for root formation to grow out a little earlier or more quickly on the lower than on the upper side of the stem or the leaf. It is a general rule in the phenomena of regeneration in *Bryophyllum* that organs which grow out first or more quickly attract for some reason the flow of sap (possibly by modifying the direction of the sap flow) and thereby inhibit or retard the growth of similar organs in other places, and this inhibitory factor is added to the influence of

[1] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 687.

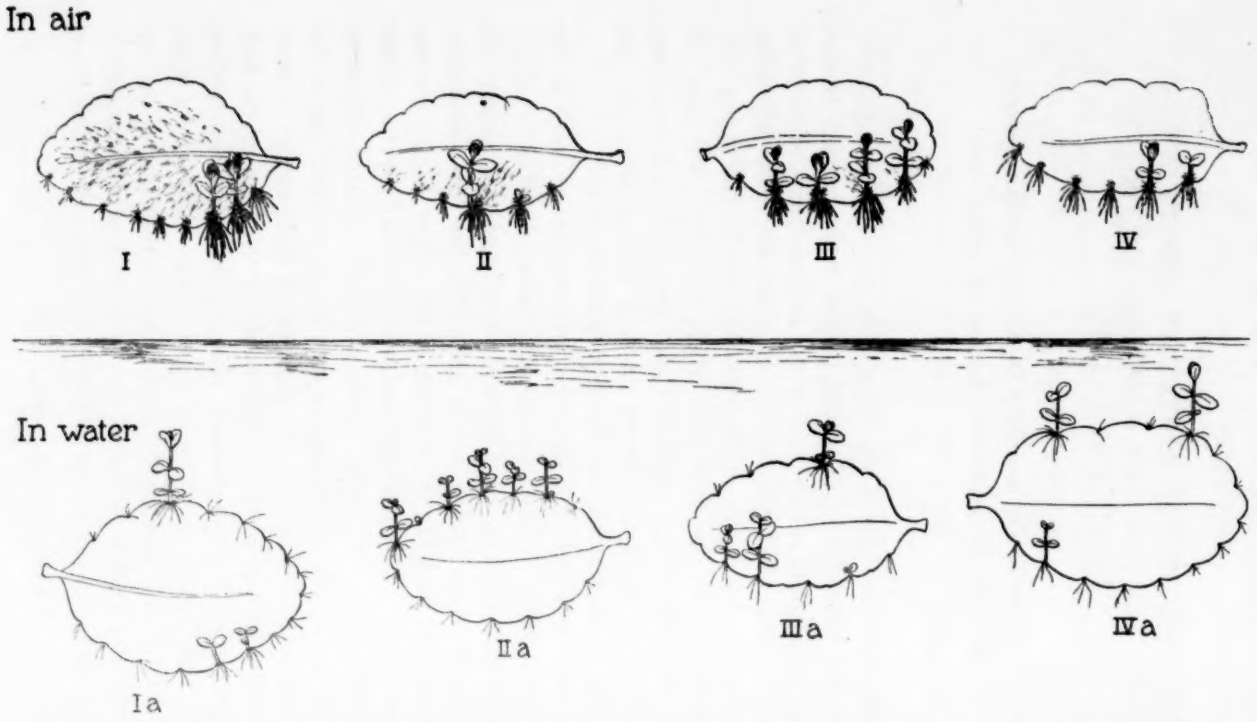

FIG. 1. Upper row, leaves suspended in air, lower row, leaves suspended in water. In moist air (upper row) roots and shoots are found only in the notches of the lower side of the leaves, while in water this directive influence of gravity does not exist, inasmuch as roots and shoots are formed on both the upper and the lower sides of each leaf. Only the leaves in the upper row show the red pigment indicated by stippling. Duration of experiment, Oct. 26 to Dec. 15, 1919.

gravity upon the distribution of sap. It is, therefore, not gravity alone which determines the directive effect in these cases but gravity in combination with the modification of the sap flow towards a growing organ.[1]

This reasoning is supported by the following facts. When we prevent the growth of roots and shoots on the lower side of the leaf by cutting off the dormant buds contained in the notches of the lower side of a leaf of *Bryophyllum* suspended vertically and sidewise, roots and shoots will now develop as abundantly on the upper side as they otherwise would have developed on the lower side; the only difference being that the roots begin to appear on the upper side slightly later than they would have appeared on the lower side.[1]

The same proof can be furnished for the formation of roots on the lower side of a horizontally placed stem. When we cut off the lower half of such a stem the roots will now form abundantly on the upper side of the stem.[1]

The fact that some sap collects on the lower side of a piece of stem suspended horizontally or on the lower side of an isolated leaf suspended sidewise in a vertical plane may be compared to the behavior of edematous liquid in animals which also follows gravity. It is possible that only that part of the sap of an isolated organ is thus affected by gravity which is not in active circulation in the vessels. The circulation in the vessels will cause an abundant collection of material at the ends of a piece of stem around the whole circumference of the piece and this may account for the fact that at the basal end of an excised piece of stem suspended horizontally in air roots develop on both the lower and upper side in such abundance that the slight quantity of sap collected through the influence of gravity on the lower side of the base becomes a negligible factor.

The real share of gravity in the directive influence on root and shoot formation is, therefore, a modest one since it needs consist only in a slightly greater collection of sap on the lower side of an organ, just sufficient to accelerate the growth of roots on the lower side.

It occurred to the writer that this conception of the directive influence of gravity on root formation might be tested in still another way; namely, by suspending leaves or pieces of stem under water. In this case the influence of gravity on root formation should dis-

appear or be greatly diminished, since the influence of the water furnished from the outside might tend to make the influence of the slight quantity of sap collected on the lower side through the influence of gravity a negligible factor.

When we suspend pieces of stem of *Bryophyllum* horizontally under water (instead of in air) roots no longer develop on the lower side only but on the upper side as well (Figs. 2 and 3). The sap will in this case collect also in somewhat greater abundance on the lower side of the stem, but this will cause no retardation of growth on the

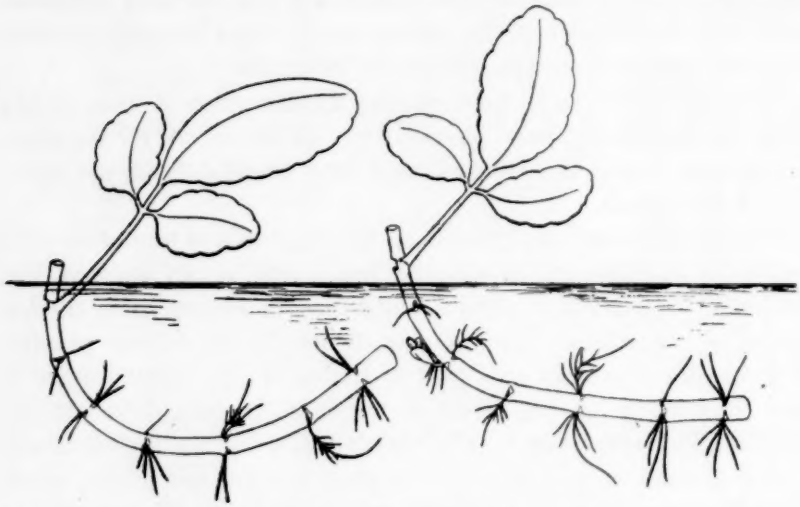

FIG. 2. Stems suspended horizontally under water. Roots form no longer exclusively on the lower side of the stems as they would have done had the stems been suspended in air, but on both the upper and lower side of the stem. The stems were originally straight and the curvature is due to geotropism; *i.e.*, greater growth on the lower side.

upper side on account of the abundance of water on the upper side of the stem. This is also true for leaves. When we suspend leaves vertically and sidewise under water the roots and shoots will develop equally fast on the upper and lower sides of the leaf and the influence of gravity will disappear (Fig. 1, lower row). In both cases it is necessary to suspend the organs near the surface of the water so that their oxygen supply will not suffer too much.

We can finally submit our idea concerning the nature of the directive effect of gravity upon the arrangement of organs to a quantitative test. When an organ (*e.g.* a leaf suspended vertically and sidewise

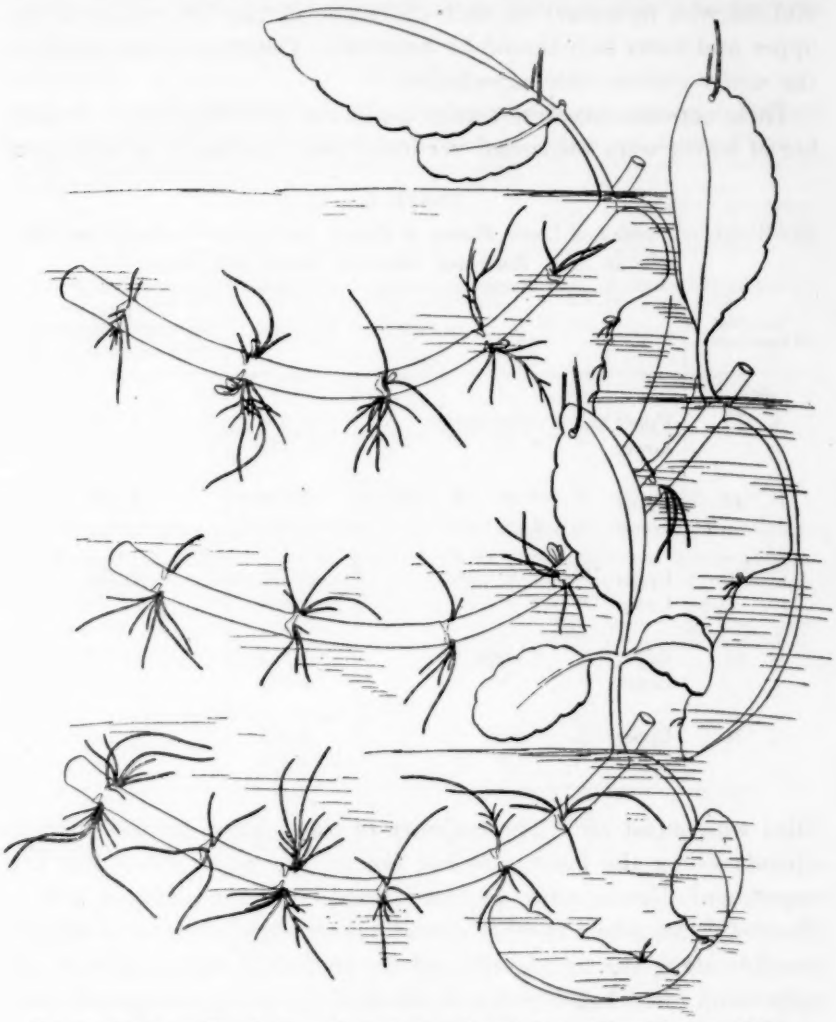

FIG. 3. Same as Fig. 2. Duration of experiment, Oct. 13 to Nov. 1, 1919.

in moist air) forms roots or shoots exclusively on the lower side, the material for these new organs must be partly withdrawn from the upper side of the organ and we must be able to prove that the dry

weight of the lower half of such an organ is always greater than the dry weight of the upper half; while when an organ forms shoots and roots on both the upper and lower side (*e.g.* a leaf suspended vertically and sidewise in water) no such difference in the dry weight of the upper and lower half should be expected. Determinations made by the writer confirm this expectation.

These experiments were carried on in the following way. A number of leaves were suspended vertically and sidewise in an aquarium

TABLE I.

Dry Weight of Upper and Lower Halves of Leaves Suspended Vertically and Sidewise in Air. Roots and Shoots on Lower Side Only.

Duration of experiment.		Dry weight.	Ratio dry weight upper halves / dry weight lower halves
days		*gm.*	
A. 22	Upper halves of six leaves.	0.620	0.815
	Lower " " " "	0.751	
B. 32	Upper " " " "	0.810	0.800
	Lower " " " "	1.011	
C. 36	Upper " " " "	0.570	0.888
	Lower " " " "	0.642	
D. 35	Upper " " eight "	0.582	0.855
	Lower " " " "	0.681	
E. 31	Upper " " five "	0.499	0.872
	Lower " " " "	0.572	

filled with moist air. The majority of these leaves formed roots in abundance on the lower side but not on the upper side. For this experiment leaves with perfect symmetry were selected. After about 4 or 5 weeks a number of such leaves were cut as accurately as possible along the middle rib and the fresh and dry weights of the upper and lower halves were determined. The dry weight of roots and tiny shoots formed on the lower side was included in the dry weight of the lower halves of the leaves. The lower halves of the leaves had, without exception, a higher dry weight, than the upper halves (Table I).

As a control the same experiments were made with leaves suspended under water which had formed roots and shoots on both their upper and their under sides though there were fewer on the upper side. In this case there was no constant difference between

TABLE II.

Controls, Dry Weight of Upper and Lower Halves of Leaves Suspended Vertically and Sidewise in Water. Roots and Shoots on Upper and Lower Sides.

Duration of experiment.		Dry weight.	Ratio $\frac{\text{dry weight upper halves}}{\text{dry weight lower halves}}$
days		*gm.*	
A. 29	Upper halves of six leaves with ten shoots.	0.561	1.04
	Lower " " " " " eleven "	0.538	
B. 32	Upper " " five " " four "	0.672	1.11
	Lower " " " " " two "	0.606	

TABLE III.

Dry Weight of Upper and Lower Halves of Leaves Suspended Vertically and Sidewise in Moist Air, Which Formed Some Shoots on Both Upper and Lower Sides.

Duration of experiment.		Dry weight.	Ratio $\frac{\text{dry weight upper halves}}{\text{dry weight lower halves}}$
days		*gm.*	
35	Upper halves of four leaves.	0.365	0.990
	Lower " " " "	0.369	
31	Upper " " seven "	0.735	0.940
	Lower " " " "	0.783	

the dry weights of the upper and lower halves. The differences found were very slight and occurred in both directions (Table II).

The same was true for leaves raised in moist air, which formed some shoots on the upper side. The dry weights of the mass of the upper and lower halves did not differ (Table III).

II.

The idea that a collection of sap occurs in the lower parts of a leaf suspended in a vertical plane can be demonstrated through the fact that leaves thus suspended in moist air form a reddish or purple pigment which has a tendency to collect in the lower parts of such a leaf. Fig. 4 shows two leaves with this pigment. The leaves had been suspended in moist air from April 17 to May 14, 1919. The

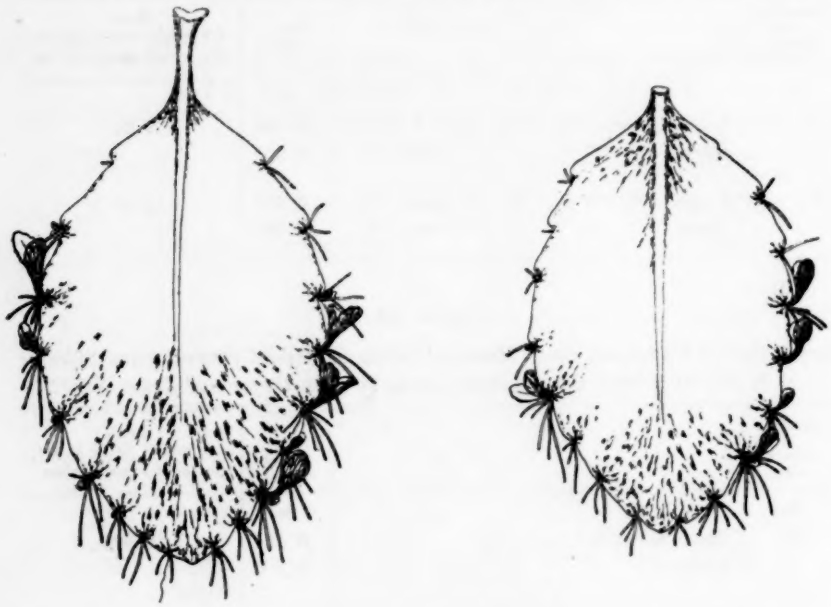

FIG. 4. Leaves suspended in moist air. Collection of reddish or purplish pigment in lower halves of the leaves, following the vessels. It is also obvious that the pigment collects more densely near each notch where a shoot or roots grow out. The leaves of the new shoots formed are full of pigment while the roots are free from it. Duration of experiment, Apr. 17 to May 14, 1919.

reddish or purple pigment is indicated in the drawing by black stippling. It is obvious that the pigment collects in the lowest parts of the leaf, that it follows the vessels, and that it also flows into the young leaves, thus supporting the view that a deflection of the sap flow towards the new shoots occurs in such a leaf. It does not,

however, collect in the new roots. A closer inspection of the leaves shows that the red pigment collects not only in the lower half of the leaves but also in those notches of the upper part of the leaf where roots or shoots are growing vigorously, thus supporting the view that the flow of sap is directed to rapidly growing organs. While a small amount of red pigment may possibly be visible in a leaf under normal conditions (especially along the edge of a young leaf), a noticeable amount is formed when the amount of water in the leaf is diminished.

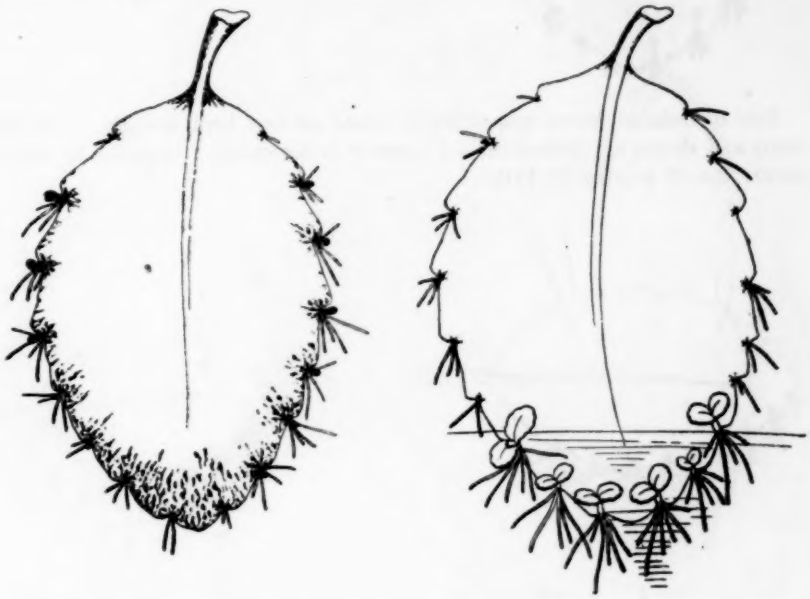

FIG. 5. Two sister leaves, one suspended in moist air, the other dipping with its apex into water. Only the former shows the pigment formation while the latter does not. This result is general and proves that the formation of the reddish pigment is favored by a diminution in the amount of water in the leaf since in the leaves suspended in moist air the ratio of dry to fresh weight is greater than in leaves which dip into water. Duration of experiment, Apr. 2 to Apr. 17, 1919.

This is shown by a comparison of the two sister leaves in Fig. 5, one of which (the one on the right) dipped with its apex into water while the other (the one to the left) was suspended in moist air. The ratio of dry weight to fresh weight of such leaves is always greater in the

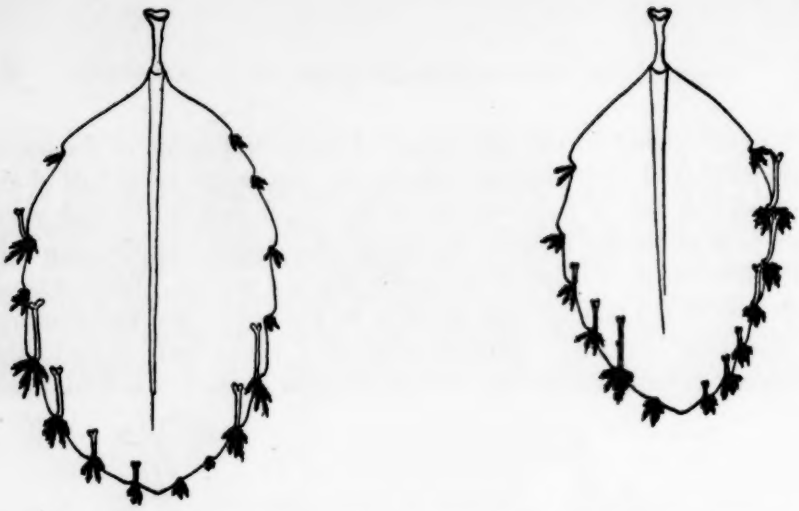

FIG. 6. Isolated leaves suspended in moist air but kept in dark. Although roots and shoots are formed no red pigment is noticeable. Duration of experiment, Apr. 20 to May 13, 1919.

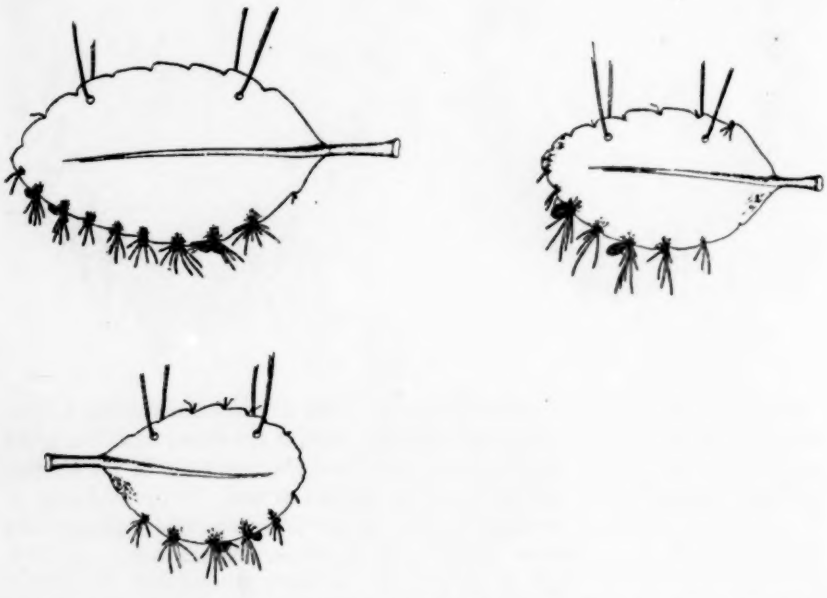

FIG. 7. Leaves suspended sidewise and in a vertical plane in moist air. The purple pigment collects near the notches on the lower side where roots and shoots develop. The young leaves are full of this purplish pigment. It collects also on the upper side of the middle rib while the lower side of the middle rib is free from pigment. In the petiole pigment exists on both the lower and upper side.

leaves suspended in air than in water. The leaf dipped into water (*i.e.* the leaf with a normal ratio of dry to fresh weight) formed no noticeable quantity of pigment while the other leaf with less water formed a considerable quantity. This difference is constant. A second factor necessary for the appearance of red pigment in the leaf is light. When leaves are suspended in moist air but kept in the dark they form no noticeable amount of red or purple pigment (Fig. 6) though they form roots and shoots.

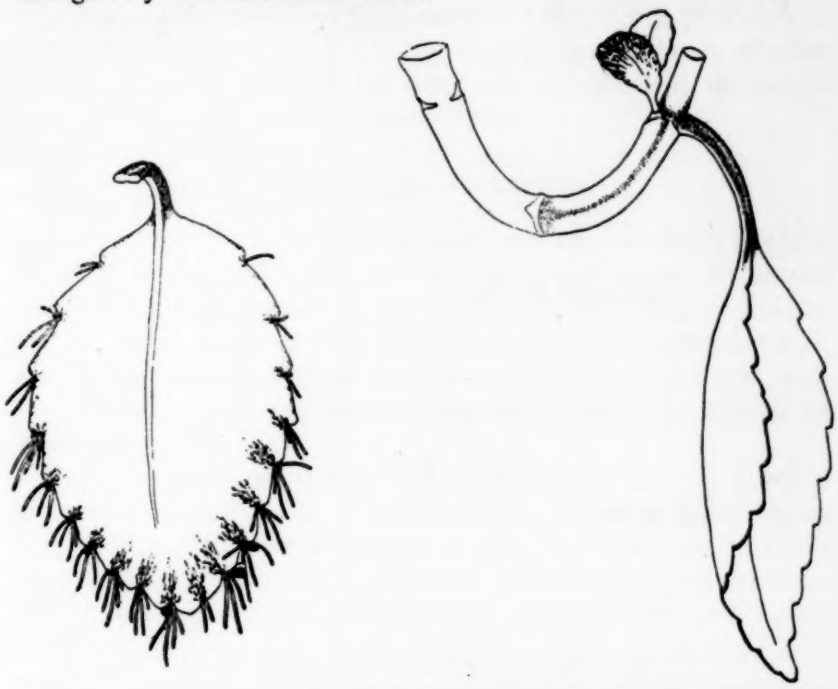

FIG. 8. Two sister leaves, one (to the right) connected with a piece of stem, the other (to the left) detached from the stem. Though both leaves are suspended in moist air only the detached (left) leaf is full of pigment, while the leaf in connection with stem is free from the purple pigment, which collects in the stem and in the leaves of the bud formed by the stem.

The most important fact for our problem is the distribution of sap in leaves suspended sidewise and in a vertical plane in moist air. In such leaves a collection of sap is found in the vessels close to the notches of the lower side of the leaves where the roots and shoots develop (Fig. 7).

We have stated in a preceding paper[2] that the sap from a leaf flows normally into the stem and that this is the reason of the inhibitory or retarding influence of the stem upon root and shoot formation in a leaf of *Bryophyllum*. This idea is supported by the fact that when a piece of stem is left in connection with a leaf suspended in moist air no collection of red or purplish pigment occurs in the leaf but that

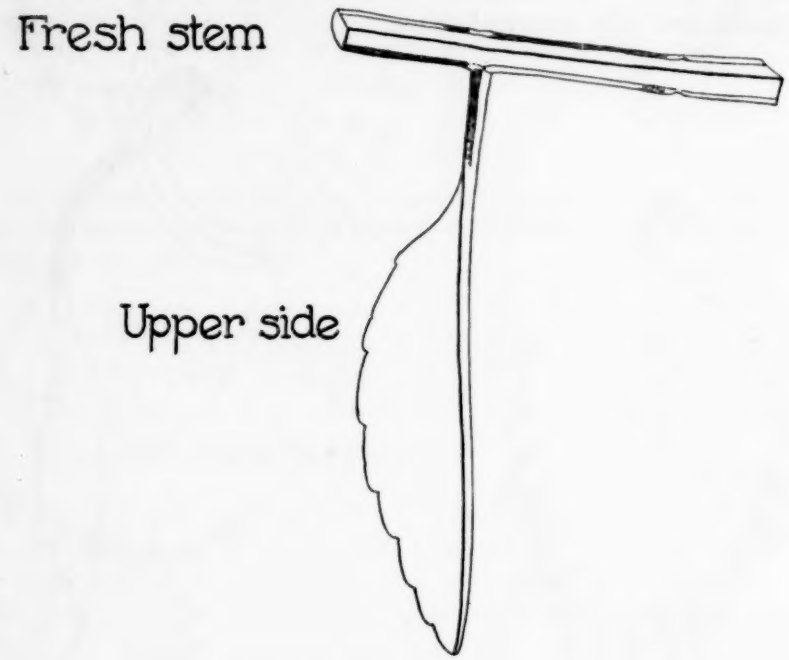

FIG. 9. Showing the distribution of the purple pigment in the stem.

it is found in the petiole, in the stem, and in the new shoots which are formed by the stem. This is illustrated by Fig. 8. The leaf on the right was left in connection with a piece of stem, while the sister leaf (to the left in Fig. 8) was detached from the stem. The latter shows a collection of the pigment in the vessels near each notch from which roots and shoots develop and the new shoots are full of the pigment. The sister leaf in connection with a piece of stem (to the right in

[2] Loeb, J., *J. Gen. Physiol.*, 1919–20, ii, 297.

Fig. 8) has no pigment in its notches, but the pigment is visible in the stem and especially in the small leaves formed on the upper side of the stem. It is needless to say that while only a few drawings are given the experiments were made on a large number of specimens, all yielding the same result. Fig. 9 shows the distribution of the pigment in the cortical layer of a piece of stem connected with a leaf.

While the purple pigment is a convenient indicator for the distribution of sap, it is not the cause of the growth of roots and shoots in a leaf. This is obvious from the fact that growth of roots and shoots takes place in isolated leaves when kept in the dark or when dipping in water, although no noticeable formation of the purple pigment takes place under these conditions.

SUMMARY AND CONCLUSIONS.

1. When leaves of *Bryophyllum calycinum* are suspended in moist air in a vertical plane and sidewise, roots and shoots are formed exclusively or predominate in the notches on the lower side of the leaves. When pieces of stems of the same plant are suspended horizontally in moist air, roots develop on the lower side of the stem, with the exception of the extreme basal end where they may develop on both sides.

2. The writer has suggested in a preceding paper that this directive influence of gravity on the arrangement of the regenerating organs may be due to the combination of two factors. The first factor is gravity, which causes a slightly greater collection of sap on the lower side of these organs, and as a consequence roots develop a little more quickly on the lower than on the upper side. The second factor is of an inhibitory character inasmuch as quite generally organs which grow out first, or which grow quickly, have a tendency to retard or inhibit the growth of similar organs in other places.

3. The writer was able to prove the action of this inhibitory factor by cutting off the lower edges of leaves suspended sidewise in a vertical plane or the lower halves of stems suspended in a horizontal plane (in moist air). In this case roots developed as abundantly on the upper side of these organs as they otherwise would have developed on the lower side.

4. It was, however, still necessary to prove the idea that gravity causes sap to collect in larger quantity in the lower parts of organs. This gap is filled by the present paper in which it is shown, first, that in the leaves suspended in moist air a red pigment is formed which has a tendency to collect gradually in the lowest parts of the leaf when the latter is suspended in a vertical plane. This red pigment serves as an indicator for the distribution of sap in the leaf and thus shows directly the tendency of the sap to collect in greater abundance on the lower edge of a leaf suspended in a vertical plane.

Second, it is shown that when leaves or stems of *Bryophyllum* are suspended, in the way described, under water instead of in moist air, roots develop on the upper side as well as on the lower side. The directive effect of gravity upon the arrangement of organs disappears in this case since the abundance of the outside water makes the effect of a slight difference in the distribution of sap between the upper and lower side a negligible factor.

Third, it is shown that the dry weight of the lower half of leaves suspended sidewise for several weeks in moist air in a vertical plane is greater than that of the upper half when roots and shoots are formed on the lower side only. This indicates that material from the upper half flows into the growing organs of the lower half. No such difference between upper and lower half of leaf is found when the leaves are suspended in the same way in water and roots and shoots are formed on both sides of the leaf.

5. It is shown that when a leaf connected with a piece of stem is suspended in moist air the red pigment goes into the stem instead of collecting in the lower part of the leaf, thus supporting the view expressed in a preceding paper that the inhibitory action of the stem on the root and shoot formation in a leaf of *Bryophyllum* is due to the fact that the material available in the leaf for organ formation is naturally sent into the stem.

ON THE CAUSE OF THE INFLUENCE OF IONS ON THE RATE OF DIFFUSION OF WATER THROUGH COLLODION MEMBRANES. I.

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(Received for publication, January 23, 1920.)

I.

When we separate a watery solution from pure water by a collodion membrane water will diffuse into the solution and solute will diffuse out. We will call this diffusion free osmosis to distinguish it from osmosis due to an outside force; *e.g.*, electrical endosmose. The free diffusion of solute into pure water occurs at a rate proportional to the concentration of the solution[1] (with the possible exception of very low or very high concentrations) and need not occupy our interest in this paper. The diffusion of water into the solution has a different character when the solute is a non-electrolyte than when it is an electrolyte. When the solute is a non-electrolyte, the initial rate of diffusion of water into the solution is (within the limit of moderate concentrations) practically a linear function of the concentration of the solute, as it should be according to the law of van't Hoff. When the solution is an electrolyte, anomalies occur which are a characteristic function of the oppositely charged ions of the electrolyte and these anomalies were described for collodion membranes in a series of papers which have appeared recently.[1, 2, 3, 4] The anomalies seem to occur only in the lower concentrations of electrolytes, below M/8 or less; above these values the osmosis seems to occur in a way similar to that observed in solutions of non-electrolytes, though this point

[1] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 717.
[2] Loeb, J., *J. Gen. Physiol.*, 1919–20, ii, 173.
[3] Loeb, J., *J. Gen. Physiol.*, 1919–20, ii, 255.
[4] Loeb, J., *Proc. Nat. Acad. Sc.*, 1919, v, 440.

needs further investigation. The present paper deals only with concentrations inside the anomalous range.

It was shown in the preceding papers that all the anomalies can be adequately described if we assume that the water in diffusing through the pores or interstices of the membrane is either positively or negatively charged and that the ions of the solution accelerate or retard the diffusion of the electrified water by their electrical charges. When we use collodion membranes which have been bathed for a short time in a 1 per cent solution of a protein (gelatin, casein, egg albumin, edestin, etc.) the effects of the two oppositely charged ions can be expressed in the following terms.

1. When we separate a neutral, alkaline, or faintly acid solution of an electrolyte with a monovalent or bivalent cation by a collodion membrane (treated with protein) from pure water, the latter diffuses into the solution as if its particles were positively charged and as if they were attracted by the anion and repelled by the cation of the solution with a force increasing with the valency of the ion and with a second constitutional quantity of the ion which we designated arbitrarily as the radius of the ion, but which needs another definition so as to include the strong effects of such monovalent ions as; *e.g.*, the aluminate or oleate anion.

2. When we separate solutions of electrolytes with a concentration of hydrogen ions of about 10^{-4} N or above, or with trivalent or tetravalent cations in sufficient concentration by a collodion membrane from pure water, water diffuses into the solution as if its particles were negatively charged and attracted by the cation and repelled by the anion of the electrolyte with a force increasing with the valency of the ion and with a second constitutional quantity of the ions still to be defined.

When we use collodion membranes *not* treated with protein one rule suffices to express all the phenomena; namely, water diffuses through the collodion membrane into the solution as if its particles were positively charged and as if it were attracted by the anion and repelled by the cation of the solution with a force increasing with the valency and a second quantity of the ion which is still to be defined.

What we designated in this statement as electrified particles of water is the watery phase or the mobile stratum of the electrical double layer (in the sense of Helmholtz) formed at the boundary of membrane and water or solution. The other stratum of this double layer, the membrane phase, must be considered as immobile in the experiments on osmosis. The influence of electrolytes on the double layer at the boundary of water and membrane will be assumed to be responsible for the phenomena of abnormal osmosis.

It follows from our previous publications that we must discriminate between two effects of electrolytes on the double layer. The one effect is the influence of certain ions on the sign of the electrification of the water or the solution phase of the double layer. Water in contact with a membrane is generally positively charged and only in the case of certain types of membranes, *e.g.* collodion membranes treated with proteins, can the sign of the charge be reversed by two kinds of ions; namely (*a*) hydrogen ions, or (*b*) simple (*i.e.* non-complex) cations whose valency is three or more.[3] All electrolytes which can lower the hydrogen ion concentration by a chemical action, *e.g.* alkalies, can restore the original positive electrification of the water stratum of the double layer. Some authors have drawn from this the conclusion that the OH ions act as directly as the H ions upon the sign of the electrification of the membrane (*e.g.* by transferring their negative charge to it). It seems to the writer that it is more in harmony with the facts to assume that the alkalies act merely by the diminution of the hydrogen ion concentration through neutralization of acid. The special effect of the two classes of positive ions—H ions and simple cations with a valency of three or above—on the sign of the electrification of the water stratum of the double layer will be discussed in another paper.

In addition to this *specific* influence of certain ions on the sign of the electrification of water at the boundary of the membrane there exists a second, more *general* effect of electrolytes on the *rate* of diffusion of water which was described in terms of electrostatic attraction and repulsion of the electrified stratum of water by the ions of the electrolyte in the previous papers, since this way of describing the results had the advantage of simplifying the presentation of the facts. It was not, however, intended to serve as a theoretical

basis for the explanation of the phenomena of abnormal osmosis. For this latter purpose we must express the facts in the following form. When we separate a solution of an electrolyte from pure water by a collodion membrane the oppositely charged ions of the electrolyte influence the initial velocity of diffusion of water through the membrane into the solution in an opposite sense; the ion with the opposite sign of charge from that of the electrified water (or the watery phase of the double layer) increasing the velocity, the ion with the same sign of charge as the watery phase of the double layer diminishing the velocity. The accelerating and retarding effects of ions were found to increase with the valency and with that other constitutional quantity which was designated as the radius of the ion but which requires further definition.

In passing we may remark that the relative retarding and accelerating effect of oppositely charged ions of an electrolyte on the rate of osmosis of water into the solution was not found to be the same for all concentrations of a solution.[2] At the lowest concentrations the effect of that ion usually (and possibly always) prevails which has the opposite sign of charge from that of the watery phase of the double layer, at a higher concentration the effect of that ion prevails which has the same sign of charge as the watery phase. Hence in the lower concentrations the accelerating effect of the electrolyte prevails over the retarding effect and for the higher concentrations the reverse is true. For a number of solutions, *e.g.* salts of monovalent cations, the turning point lies at a concentration of about M/256. The anomalous osmosis ceases at that concentration of the solution where the retarding and accelerating effects of the oppositely charged ions become equal. From then on the solutions of electrolytes seem to behave like those of non-electrolytes. This group of facts has been described in a preceding paper[2] and will not be discussed here.

In our experiments on free osmosis the collodion membrane was bounded on one side by pure water and on the other by the solution. When both sides of the membrane are bounded by identical solutions the rate of diffusion of water and of electrolyte in opposite directions is equal, and no change occurs. When, however, an external difference of potential is produced on the two sides of the

membrane, a transport of water or of liquid occurs through the membrane towards that electrode whose sign of charge is the opposite of that of the watery phase of the double layer in the pores or interstices of the membrane. This is the well known phenomenon of electrical endosmose which was first investigated experimentally by Quincke and Wiedemann and which was explained mathematically by Helmholtz. The earlier workers found that the watery phase of the double layer was generally positively electrified. Perrin[5] made the remarkable discovery that in the case of certain diaphragms, such as powdered charcoal, carborundum, gelatin, etc., the sign of charge can be reversed at will, chiefly with the aid of acid and of alkali. In a slightly acid medium the liquid moves to the anode, in a slightly alkaline medium it moves to the cathode. This has been confirmed by every observer, and his deductions have been generally accepted.

It has been suggested by Girard, Bernstein, Bartell and Hocker, and Freundlich[6] that the cases of so called negative osmosis where liquid diffuses from acid into pure water instead of in the opposite direction might be in reality manifestations of electrical endosmose. The only difference between the case of free osmosis and electrical endosmose being, according to these authors, the source of the potential difference, which is an external one in the case of electrical endosmose and an internal one—*e.g.* a diffusion or a boundary potential—in the case of free osmosis. But this is thus far merely an hypothesis which is not yet adequately supported by facts.

The possibility of correlating the phenomena of free and electrical osmosis meets at present with a difficulty. Our experiments on collodion membranes leave no doubt that in the case of *free osmosis* the influence of electrolytes on the velocity of diffusion of water from pure solvent to solution through the collodion membrane is an addi-

[5] Perrin, J., *J. chim. physique*, 1904, ii, 601; 1905, iii, 50.

[6] Girard, P., *Compt. rend. Acad.*, 1908, cxlvi, 927; 1909, cxlviii, 1047, 1186; 1910, cl, 1446; 1911, cliii, 401; La pression osmotique et le mécanisme de l'osmose, Publications de la Société de Chimie-physique, Paris, 1912. Bernstein, J., Elektrobiologie, Braunschweig, 1912. Bartell, F. E., *J. Am. Chem. Soc.*, 1914, xxxvi, 646. Bartell, F. E., and Hocker, C. D., *J. Am. Chem. Soc.*, 1916, xxxviii, 1029, 1036. Freundlich, H., *Kolloid-Z.*, 1916, xviii, 11.

tive effect of the two oppositely charged ions. Perrin,[5] however, states that in the case of electrical endosmose only one of the oppositely charged ions of an electrolyte influences the transport of liquid through the membrane; namely, the one with the same sign of charge as that of the water (or with the opposite sign of charge from that of the membrane).

II.

Perrin's[5,7] view of the influence of electrolytes on the amount of liquid transported in electrical endosmose rests on the assumption that the sign of the electrification of the double layer is primarily determined by the H and OH ions. He assumes that the positive electrification of a membrane bounded by liquid containing a monovalent acid is due to the adsorption of a layer of hydrogen ions by the membrane. This membrane layer of adsorbed hydrogen ions is the fixed stratum of the double layer and the next stratum of the liquid—the watery phase—contains a corresponding excess of negative ions. The stratum of negative ions is sufficiently far removed from the fixed layer so as to be able to undergo the tangential displacement on which the phenomena of electrical osmosis are supposed to depend.

Perrin assumes that the negative electrification of a membrane under the influence of a monovalent base is due to the OH ions situated in that stratum of the liquid which is in immediate contact with the membrane, while a corresponding excess of positive ions exists in the opposite stratum of liquid (the watery phase, in our terminology). When another electrolyte is added to a weak acid or weak alkaline solution the charge of the membrane is, according to Perrin, *influenced only by one of the two ions* of the electrolyte added; namely, the one with the opposite sign of charge from that of the membrane.

"When a liquid electrifies a membrane with a certain sign the addition to this liquid of a polyvalent ion of the same sign does not increase the electrification, while the addition of a polyvalent ion of the opposite sign diminishes this electrification considerably. The influence of bivalent ions is inferior to that of trivalent ions and that of trivalent ions is inferior to that of tetravalent ions. In the case of the ions with high valencies the diminution of the charge can result in the complete reversal of the sign of the charge."

[7] Perrin, J., Notice sur les titres et travaux scientifiques de M. Jean Perrin, Paris, 1918, 36–37.

His theory which has revolutionized colloid chemistry is expressed in the following statement.

"The primary factor of this electrification is always the action of the hydrogen or the hydroxyl ions, which are pressed against the membrane in the same way, no matter whether polyvalent ions are present or absent. But if polyvalent ions of the opposite sign are present they are attracted towards the membrane. To be more precise, let us suppose a liquid with a monovalent acid; hydrogen ions cover the membrane with a positive charge according to the degree of acidity. Behind them, at a distance which results from an equilibrium between the osmotic and the electric forces are found the monovalent negative ions forming the second stratum of the double layer. If we now add negative polyvalent ions, *e.g.* $Fe(CN)_6$, the osmotic forces acting on the tetravalent $Fe(CN)_6$ remain of the same order as before while the electric force is multiplied by four; the density of the double layer will therefore diminish and as a consequence the P.D. of contact and the amount of electrical endosmose. It only remains to explain in a precise manner reversion of the sign of charge due to the presence of the necessary amount of $Fe(CN)_6$ ions."[7]

Perrin's view on the effect of electrolytes on the double layer does not agree with our experiments with collodion membranes which show unequivocally that the influence of electrolytes on the rate of diffusion of water in the case of free osmosis is an additive effect of the two oppositely charged ions of an electrolyte, and not the effect of only one of the two ions.

If we assume that the influence of ions is the same in the case of free osmosis and in electrical endosmose an increase in the valency of the anion, according to Perrin, should not increase the rate of diffusion of positively electrified water in free osmosis, since in this case the membrane has the same sign of charge as the anion. A glance at Fig. 1 shows, however, that when we separate a solution from pure water by a collodion membrane the initial rate of diffusion of water into the solution increases in the lower concentrations of different potassium salts with increasing valency of the anion when the water is positively charged. In this case a watery solution of one of these salts was put into a collodion bag connected with a manometer and the bag was put into a beaker containing distilled water. The solutions of NaCl, $CaCl_2$, Na_2SO_4, and $Na_4Fe(CN)_6$ were rendered alkaline by dissolving the neutral salt in M/1,024 or M/1,000 KOH. The abscissæ are the logarithms of the concentration and the ordinates are the rise in the level of solution in the manometer after 20 minutes.

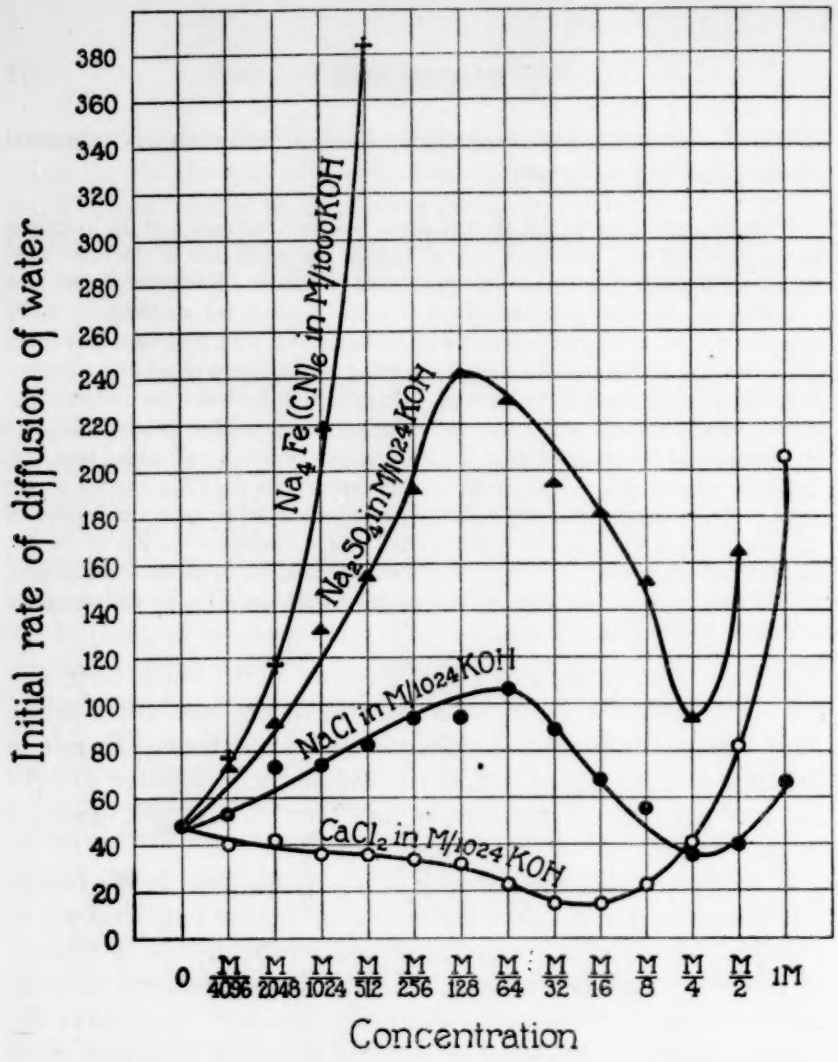

Fig. 1. Initial rate of diffusion of pure water through a collodion membrane into a solution containing an electrolyte. Abscissæ are the logarithms of concentration, ordinates rise of level of water in manometer connected with solution after 20 minutes. Solutions of salts were all rendered alkaline by enough KOH to make the solution about 10^{-3} N in regard to KOH. The water was positively electrified. The curves show that the initial rate of diffusion of water into the solution increases with increasing valency of the anion of the electrolyte, though the membrane has the same sign of charge as the anion. The cations have a depressing effect, increasing also with the valency. The drop in the curve beyond a concentration of M/128 or M/64 is due to the fact that beyond this concentration the effect of the cation begins to prevail over that of the anion, until at a concentration of M/8 or M/4 the gas pressure effect of the electrolyte begins to prevail over the electrical effect.[2, 3, 8]

[8] Loeb, J., *J. Gen. Physiol.*, 1919–20, ii, 273.

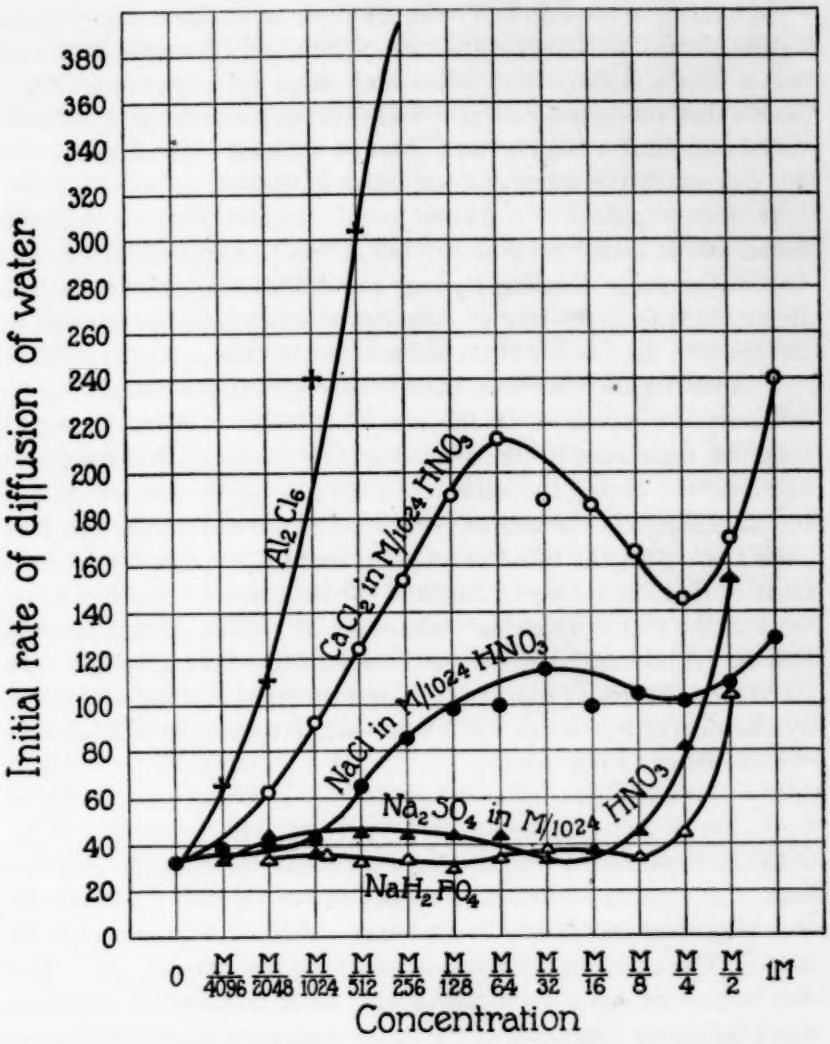

FIG. 2. Initial rate of diffusion of water from pure water through collodion membrane to solution of electrolytes rendered about 10^{-3} N acid through addition of HNO_3. Though the water is negatively (and the membrane positively) electrified the initial rate of diffusion of water into the solution increases with increasing valency of the cation and diminishes with increasing valency of the anion. The drop in the curves in concentrations beyond M/64 or M/32 is due to the fact that in concentrations higher than these the depressing effect of the anion prevails over the opposite effect of the cation. For explanation of second rise of curve see legend of Fig. 1.

Moreover, according to Perrin, an increase in the valency of the cation should not increase the rate of diffusion of water from pure water into a slightly acid solution of salts, yet a glance at Fig. 2 shows that the initial rate of diffusion of water through a collodion membrane into a slightly acid solution increases considerably with the increase in the valency of the cation of the salt added.

In former papers,[1, 2, 3] further proof of these statements can be found and in addition the writer has shown that in neutral solutions (where the water diffusing through the collodion membrane is positively electrified) the rate of diffusion of water into the solution increases with the valency of the anion of the electrolyte in the solution.

This discrepancy between the actual observations concerning the influence of electrolytes on the rate of diffusion of water through a collodion membrane in free osmosis on the one hand, and the theory of Perrin concerning the influence of electrolytes on the rate of diffusion of liquid in the case of electrical endosmose on the other indicates that either the influence of electrolytes is not the same in both cases or that the theory of Perrin is not the correct expression of the facts in the case of electrical endosmose, at least for collodion membranes. It seemed, therefore, necessary to test the influence of electrolytes on the rate of transport of water through collodion membranes by electrical endosmose as a first step towards a theory of the influence of electrolytes on free osmosis.

III.

In the experiments on electrical endosmose we used the collodion bags which served for the experiments on free osmosis. These collodion bags were cast inside an Erlenmeyer flask of a volume of about 50 cc. The collodion flask was closed with a rubber stopper which was perforated by a glass rod serving as a manometer. The bag was filled at the beginning of the experiment with the solution whose influence on the osmotic transport was to be investigated and was put into a beaker containing the identical solution. The bag was then so adjusted that the upper level of the rubber stopper was at the surface of the liquid in the beaker and that a column of liquid of about 30 mm. in the manometer was above the level of the liquid in the beaker. The manometer was a glass tube with a bore of about 2

mm. in diameter. One platinum electrode was put into this glass tube and one into the beaker. The distance between the electrodes was approximately the same in all experiments; namely, 6.7 cm. The electrode in the manometer was usually that pole towards which the transport of liquid occurred so that the rise of level in the manometer could serve as a measure for the volume of liquid transported. In this case the transport occurred against a hydrostatic pressure and it was necessary to compare the rise of level at about the same pressure head in different experiments.

The volume of liquid transported is a function of the external potential difference which was either 50 or 40 volts in our experiments. It turned out that in these experiments a disturbing variable entered inasmuch as with constant voltage the intensity of the current rose slowly and with the rise in intensity the amount of liquid flowing to one of the poles also rose gradually. The writer is inclined to interpret this gradual increase in intensity of current as being due to a gradual increase in the number of interstices through which the current can flow; and this means also an increase in the number of capillary spaces through which electrical endosmose can occur. Hence, for measurements of the transport of liquid only those changes in level could be used which occurred after the current had become approximately constant. In order to accelerate this process at the beginning of each experiment, a P.D. of 200 volts was used for 2 minutes or less until the intensity of the current was sufficiently high (above 1.0 or 2.0 milliamperes), and then the P.D. was lowered to the 40 or 50 volts desired. The voltage was then kept constant. As a measure of the effect of an electrolyte on the volume of liquid transported we used the rise in the level of liquid in the glass tube during the first 15 minutes after the current had become fairly constant. The following records will illustrate the way our figures were obtained. We omit the preliminary short treatment of the solution with a current of 200 volts, and give only the records for the 50 volt effects. The solutions used were M/512 KCl, M/512 K_2SO_4, M/512 and M/1,024 $K_4Fe(CN)_6$, M/512 $CaCl_2$, and M/512 $BaCl_2$. The solutions were almost neutral but slightly on the acid side of neutrality, the pH being about 6.2, and the transport of liquid occurred towards the cathode which was put into the glass tube. Table I gives the time in minutes, the in-

TABLE I.

Transport of Liquid in Electrical Endosmose in Approximately Neutral Solutions, pH = 6.2. 50 Volts. Cathode in Capillary.

Solution.	Time.	Milliampere.	Rise of level of liquid in manometer.
	min.		*mm.*
	0	1.3	+1.0
	5	1.7	+2.0
	10	1.9	+2.5
M/512 KCl	15	2.1	+3.5
	20	2.15	+5.0
	25	2.18	+6.0
	30	2.18	+7.0
	0	1.0	+1.0
	5	1.4	+1.5
	10	1.9	+2.0
	15	2.4	+4.0
M/512 K_2SO_4	20	2.3	+6.0
	25	2.3	+8.0
	30	2.25	+10.5
	35	2.25	+12.0
	40	2.26	+14.0
	45	2.2	+16.0
	0	1.0	+1.5
	5	1.45	+7.5
	10	2.0	+16.0
	15	2.35	+26.0
M/1,024 $K_4Fe(CN)_6$	20	2.45	+36.0
	25	2.45	+43.0
	30	2.6	+48.0
	35	2.7	+54.0
	40	2.76	+60.0
	0	1.4	+1.0
	5	2.4	+6.5
	10	3.4	+14.0
	15	3.85	+25.0
M/512 $K_4Fe(CN)_6$	20	4.0	+33.0
	25	4.2	+39.0
	30	4.2	+45.0
	35	4.2	+52.0
	40	4.4	+59.0

TABLE I—*Concluded.*

Solution.	Time.	Milliampere.	Rise of level of liquid in manometer.
	min.		mm.
	0	1.8	+3.0
	5	1.8	+3.0
	10	1.7	+3.0
M/512 $CaCl_2$	15	1.55	+2.0
	20	1.5	+1.5
	25	1.5	0
	30	1.5	−1.0
	0	2.3	0
	5	1.6	−1.5
	10	1.2	−3.0
M/512 $BaCl_2$	15	1.15	−4.5
	20	1.15	−5.5
	25	1.1	−6.5
	30	1.15	−7.5

tensity of the current in milliamperes, and the rise in the level of the liquid in the glass tube.

In the case of M/512 KCl the current became approximately constant after 15 minutes (2.1 to 2.2 milliamperes) and the rise in the level of liquid in the glass tube between 15 and 30 minutes, *i.e.* 7 − 3.5 mm. = 3.5 mm., was used as a measure for the relative influence of M/512 KCl (approximately neutral) upon the rate of endosmotic transport of liquid.

The figures for transport during 15 minutes were selected as follows: for M/512 $K_2SO_4 = 10.5 - 4 = 6.5$; for M/512 $K_4Fe(CN)_6 = 45 - 25 = 20$; etc.

Since in the case of M/512 $K_4Fe(CN)_6$ the intensity of the current was greater than in the experiments with KCl or K_2SO_4, an experiment with M/1,024 $K_4Fe(CN)_6$ is added in the table. It gave 48 − 26 = 22 mm. as the transport number though the intensity of current was almost as low as in the case of M/512 K_2SO_4 or M/512 KCl.

Experiments with neutral solutions of salts with bivalent cation, like $CaCl_2$, $BaCl_2$, gave no transport with electrical endosmose even with 100 volts and an intensity of current of 4.8 milliamperes. Table II gives the transport numbers for various approximately neutral solutions.

Table II shows that the rate of endosmotic transport increases in almost neutral solutions of salts with increasing valency of the anion and diminishes with increasing valency of the cation. Electrolytes influence, therefore, the osmotic transport in exactly the same sense in free and in electrical osmosis. This influence is in both cases an additive effect of the oppositely charged ions of the electrolyte. In the presence of the salt mentioned the watery phase of the double layer is positively charged. The slight fall of level in the case of $CaCl_2$ and of $BaCl_2$ must be ascribed to the pressure head of about 30 mm. solution existing at the beginning of the experiment; since the same or a slightly more rapid fall of level occurs if no current passes through the liquid.

TABLE II.

Relative Transport of Liquid by Electrical Endosmose in Approximately Neutral Solutions, pH = 6.2. 50 Volts.

	Rise of level of liquid in manometer in 15 min.
	mm.
M/512 KCl	3.5
M/512 K_2SO_4	6.5
M/512 $K_4Fe(CN)_6$	20.0
M/1,024 $K_4Fe(CN)_6$	22.0
M/512 $CaCl_2$	0
M/512 $BaCl_2$	0

We will now show that the statements made for neutral solutions are also true for alkaline solutions. In alkaline solutions the watery phase of the double layer is also positively charged and the liquid is transported to the cathode as in the case of neutral solutions. The cathode was put into the glass tube and the rise of level in the glass tube during the first 15 minutes after the current had become fairly constant was used as a measure for the transport. The solutions were brought to the same alkalinity as that of M/1,000 KOH and the pH varied between 10.9 and 11.0 (Table III).

The result is the same as before: the rate of endosmotic transport increases in alkaline solutions with increasing valency of the anion and diminishes with increasing valency of the cation. Electrolytes

influence, therefore, the transport of liquid in alkaline solution in the same sense in electrical endosmose as in free osmosis. This influence is in both cases an additive effect of the oppositely charged ions of the electrolyte. In the alkaline solutions the watery phase of the double layer is positively charged as it is in neutral solutions. If Perrin's rule applied to these experiments, the increasing valency of the anion should have had no effect.

Table III contains also the transport numbers of solutions of Na acetate, Na aluminate, and K oleate which are all higher than those of NaCl, although the anion is monovalent in each case. Solutions of

TABLE III.

Transport of Liquid by Electrical Endosmose to the Cathode in Alkaline Solutions, pH = 10.9 to 11.0. 40 Volts.

	Milliampere.	Rise of level of liquid in manometer in 15 min.
		mm.
M/512 NaCl	1.1	3.5
M/512 Na_2SO_4	1.6	10.0
M/512 $Na_4Fe(CN)_6$	2.1	22.0
M/512 $CaCl_2$	1.2	0
M/512 $BaCl_2$	1.15	0
M/512 Na acetate	1.35	7.0
M/512 $NaAlO_2$	1.15	7.0
M/512 K oleate (pH = 9.4)	1.15	22.5

these salts also attract water more powerfully than solutions of NaCl in the case of free osmosis and the influence of these salts in electrical endosmose is parallel to their influence in free osmosis. These salts illustrate the statement that in addition to the valency another constitutional quantity of the ions determines their influence on the transport of liquid in free and electrical endosmose.

We finally investigated the electrical transport of liquid in acid solutions. The salt solutions were made N/1,000 acid by the addition of HNO_3; the pH was in all cases exactly 3.0. Table IV gives the results. The anode was in the glass tube. In this case it was necessary to use membranes which had received a gelatin treatment.

If Perrin's rule applied to these cases, the increasing valency of the cation should not have influenced the result in these acid solutions.

We notice, however, that the rate of endosmotic transport to the anode increases in acid solutions with the increase in the valency of the cation and diminishes with the increase in the valency of the anion. Electrolytes influence, therefore, the transport of liquid in acid solutions in the same sense in the case of free and of electrical osmosis. The influence is in both cases an additive effect of the oppositely charged ions of the electrolyte. The watery phase of the electrical double layer is negatively charged in acid solutions of the hydrogen ion concentration used in this case; namely, 10^{-3} N.

TABLE IV.

Relative Transport of Liquid in Electrical Endosmose to the Anode in Acid Solutions, pH = 3.0. 40 Volts.

	Milliampere.	Rise of level of liquid in manometer in 15 min.
		mm.
M/512 NaCl	3.7	5.5
M/512 $CaCl_2$	2.6	11.5
M/512 $BaCl_2$	4.0	13.0
M/512 $CeCl_3$	3.5	16.5
M/512 $ThCl_4$	3.8	18.0
M/512 Na_2SO_4	3.4	0
M/512 Na_2 oxalate	3.2	0
M/512 NaH_2PO_4	1.8	2.0

If we summarize all three cases we may state that in both free and electrical osmosis, the transport of liquid is accelerated by that ion of an electrolyte which has the opposite sign of charge as the watery phase of the double layer (or the same sign of charge as the collodion membrane) and retarded by that ion which has the same sign of charge as the watery phase of the double layer (or the opposite sign of charge as the collodion membrane); and that both the accelerating and the retarding effect of ions increase with their valency and a second constitutional quantity of the ion which is still to be defined and for which the high transport number of several salts in Table III may serve as an example.

The writer has made a number of experiments on electrical endosmose with different concentrations of electrolytes. The curves repre-

senting this influence on the transport of liquid in electrical endosmose through collodion membranes seem to be similar to the curves, representing the influence of different concentrations of the same electrolytes on free osmosis, which were published in a preceding paper.[2]

In experiments with concentrations of electrolytes above M/512 or M/256 it is wiser to work with a lower voltage to avoid the excessive development of gas bubbles. Table V gives the numbers for the rise of liquid in the manometer for different concentrations of $K_4Fe(CN)_6$

TABLE V.

Influence of Concentration on Transport of Liquid by Electrical Endosmose to Cathode. 20 Volts.

	Milliampere.	Rise of level of liquid in manometer in 15 min.
		mm.
M/2,048 $K_4Fe(CN)_6$	0.1	1.5
M/1,024 $K_4Fe(CN)_6$	0.5	9.0
M/512 $K_4Fe(CN)_6$	0.9	10.5
M/256 $K_4Fe(CN)_6$	1.2	6.0
M/128 $K_4Fe(CN)_6$	1.8	3.0
M/64 $K_4Fe(CN)_6$	3.0	0

during 15 minutes after the intensity of the current had become fairly constant. The P.D. applied was 20 volts.

The maximum of transport of liquid in electrical endosmose was reached at a concentration of about M/512 $K_4Fe(CN)_6$ and then the electro-endosmotic transport fell rapidly to zero with increasing concentration, although the intensity of the current increased with concentration. The drop in the curves representing the initial rate of diffusion of water from pure water to solution through collodion membranes in the case of free osmosis is therefore paralleled in the case of electrical endosmose (Table V). A fuller account of these results will shortly be published.

Theoretical Remarks.

According to the formula of Helmholtz for the transport of liquid by a current through capillaries, modified by Perrin,[9] we have

$$v = \frac{q \cdot \epsilon \cdot E \cdot D}{4 \pi \cdot \eta \cdot l}$$

where v is the quantity of liquid carried electro-osmotically, ϵ is the potential difference between the two strata of the double layer, E the external electromotive force, D the dielectric constant of the medium, η the coefficient of internal friction, and l the distance of the external electrodes. Since in our experiments all quantities occurring in this formula except v and ϵ were kept approximately constant, we must attribute the influence of electrolytes on the quantity of transport v to an influence of the ions on ϵ. We must therefore conclude that the influence of electrolytes on the rate of free osmosis is due to the effect of the ions of the electrolyte on the quantity of charge on the unit area of the Helmholtzian double layer. Our experiments on both free and electrical osmosis show that this influence is an additive effect of the two oppositely charged ions of the electrolyte at least in the case of collodion membranes. Since the quantity of transport v increases with the value of ϵ we must further conclude that the ion with the same sign of charge as the watery phase of the double layer diminishes the value of ϵ since this ion diminishes transport in both free and in electrical osmosis; while the ion with the opposite sign of charge as this watery phase increases the value of ϵ in both forms of osmosis. Both effects increase with the valency and with the second constitutional quantity of the ion (Table III). The total effect of the two oppositely charged ions of an electrolyte on the rate of diffusion of water through a collodion membrane is therefore the difference between the opposite effects of its ions on the value of ϵ. These statements give the theoretical basis of what we called in our former papers the apparent electrostatic action of the ions on the rate of diffusion of the electrified particles of water from pure water into solution through a collodion membrane (free osmosis).

[9] Freundlich, H., Kapillarchemie, Leipsic, 1909, 226.

Positively charged particles of water in the pores or interstices of the membrane will be driven to that side of the membrane which is more negatively charged. Since this is usually the solution side, water will be driven from the side of pure water into the solution.

When the collodion membrane has been treated with a protein, it is also generally negatively charged when bounded by water except when the solution contains hydrogen ions or simple trivalent or tetravalent cations beyond a certain concentration (which for H is 10^{-4} N); in this case the membrane is positively and the watery phase is negatively charged. If we add in this case an electrolyte to the water, the charge on the membrane is increased by the cations and diminished by the anions of the electrolyte. Whenever the positive charge on the solution side of the membrane is greater than on the opposite side, the negatively charged particles of water will diffuse from the side of pure water to the side of solution.

When the charge on the solution side of the membrane is diminished by the electrolyte so that the charge is smaller than on the side of pure water, the liquid will flow through the membrane from solution side to the side of pure water (negative osmosis).

Our experiments were made with collodion membranes only and it is possible that Perrin's statement holds for other types of membranes. It seems, however, that in the case of the influence of electrolytes on the value of ϵ at the boundary of oil drops and water the effect is also an additive one of the oppositely charged ions. Powis[10] has measured this value from the velocity of the motion of oil drops through solutions on the basis of the Helmholtz-Perrin formula for five electrolytes, KCl, $BaCl_2$, $AlCl_3$, $ThCl_4$, and $K_4Fe(CN)_6$. The oil particles are negatively charged and their charge is increased more by $K_4Fe(CN)_6$ than by KCl. The charge is diminished with the increasing valency of the cation. This indicates that the influence of electrolytes on the value of the potential difference of the double layer is in this case also an additive effect of the two kinds of ions.

We have stated that in the case of free osmosis the rate of diffusion of water from pure solvent to solution through a collodion membrane increases at first with increasing concentration, reaches a maximum

[10] Powis, F., *Z. physik. Chem.*, 1915, lxxxix, 91.

(which for many electrolytes lies at a concentration of about M/256) and then drops again with a further increase in concentration. It seems from the writer's experiments that the same phenomenon occurs in the case of electrical endosmose through collodion membranes and that the turning point lies near M/512. Powis reports a similar effect of concentration in his observation on the motion of oil drops in an electrical field, and recently published experiments of Kruyt[11] on "current potentials" demonstrate the same phenomenon.

It seems to follow from this that the density of the electrical double layer at the boundary of watery phase and membrane increases at first with increasing concentration of an electrolyte up to a certain point which for a number of electrolytes seems to lie at about M/512. If the concentration of the electrolyte rises beyond this point, the density of the charge on the double layer diminishes rapidly with a further increase in the concentration of the electrolyte.

SUMMARY.

1. In three previous publications it had been shown that electrolytes influence the rate of diffusion of pure water through a collodion membrane into a solution in three different ways, which can be understood on the assumption of an electrification of the water or the watery phase at the boundary of the membrane; namely,

(*a*) While the watery phase in contact with collodion is generally positively electrified, it happens that, when the membrane has received a treatment with a protein, the presence of hydrogen ions and of simple cations with a valency of three or above (beyond a certain concentration) causes the watery phase of the double layer at the boundary of membrane and solution to be negatively charged.

(*b*) When pure water is separated from a solution by a collodion membrane, the initial rate of diffusion of water into a solution is accelerated by the ion with the opposite sign of charge and retarded by the ion with the same sign of charge as that of the water, both effects increasing with the valency of the ion and a second constitutional quantity of the ion which is still to be defined.

[11] Kruyt, H. R., *Kolloid-Z.*, 1918, xxii, 81.

(*c*) The relative influence of the oppositely charged ions, mentioned in (*b*), is not the same for all concentrations of electrolytes. For lower concentrations the influence of that ion usually prevails which has the opposite sign of charge from that of the watery phase of the double layer; while in higher concentrations the influence of that ion begins to prevail which has the same sign of charge as that of the watery phase of the double layer. For a number of solutions the turning point lies at a molecular concentration of about M/256 or M/512. In concentrations of M/8 or above the influence of the electrical charges of ions mentioned in (*b*) or (*c*) seems to become less noticeable or to disappear entirely.

2. It is shown in this paper that in electrical endosmose through a collodion membrane the influence of electrolytes on the rate of transport of liquids is the same as in free osmosis. Since the influence of electrolytes on the rate of transport in electrical endosmose must be ascribed to their influence on the quantity of electrical charge on the unit area of the membrane, we must conclude that the same explanation holds for the influence of electrolytes on the rate of transport of water into a solution through a collodion membrane in the case of free osmosis.

3. We may, therefore, conclude, that when pure water is separated from a solution of an electrolyte by a collodion membrane, the rate of diffusion of water into the solution by free osmosis is accelerated by the ion with the opposite sign of charge as that of the watery phase of the double layer, because this ion increases the quantity of charge on the unit area on the solution side of the membrane; and that the rate of diffusion of water is retarded by the ion with the same sign of charge as that of the watery phase for the reason that this ion diminishes the charge on the solution side of the membrane. When, therefore, the ions of an electrolyte raise the charge on the unit area of the membrane on the solution side above that on the side of pure water, a flow of the oppositely charged liquid must occur through the interstices of the membrane from the side of the water to the side of the solution (positive osmosis). When, however, the ions of an electrolyte lower the charge on the unit area of the solution side of the membrane below that on the pure water side of the membrane, liquid will diffuse from the solution into the pure water (negative osmosis).

4. We must, furthermore, conclude that in lower concentrations of many electrolytes the density of electrification of the double layer increases with an increase in concentration, while in higher concentrations of the same electrolytes it decreases with an increase in concentration. The turning point lies for a number of electrolytes at a molecular concentration of about M/512 or M/256. This explains why in lower concentrations of electrolytes the rate of diffusion of water through a collodion membrane from pure water into solution rises at first rapidly with an increase in concentration while beyond a certain concentration (which in a number of electrolytes is M/512 or M/256) the rate of diffusion of water diminishes with a further increase in concentration.

THE EFFECT OF TEMPERATURE UPON FACET NUMBER IN THE BAR-EYED MUTANT OF DROSOPHILA.*

PART I.

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(Received for publication, December 30, 1919.)

INTRODUCTION.

Environment plays an important part in the development of every organism. In a few cases, it becomes a determinative agent in that particular structures are developed only under special external stimuli.

The bar-eyed mutant of *Drosophila melanogaster* Meig (*ampelophila*) shows such a response to temperature. Primarily this germinal factor produces a reduction in the number of facets in the compound eyes. Under a constant environment, it produces practically a constant effect in all individuals. Under varied temperature conditions, the amount of reduction varies inversely with the temperature.

The present study[1] is an attempt to work out these relations in detail. From the standpoint of the modern physiologist, the results are of particular interest in demonstrating in the same living material a physiological reaction whose rate is an exponential function of the temperature, while another reaction has a rate which is clearly a linear function of the temperature.

From the standpoint of experimental embryology in its broadest sense, this study gives an evaluation of some of the external and internal factors involved in the development of a particular structure,

* Contribution from the Zoological Laboratory of the University of Illinois, No. 148.

[1] This investigation was developed under the direction of Dr. Charles Zeleny. The study was made possible through the excellent equipment for environmental control installed at the Vivarium.

the compound eye. Furthermore, the particular stages in development at which the facet-determining reaction is initiated has been ascertained by the use of the temperature differences.

Materials and Methods.

The bar-eyed mutant of *Drosophila* was first described by Tice. Considerable variation was noticed in this character. Zeleny and Mattoon, and May have shown that part of this variation was due to germinal differences. It was noted, however, that environmental factors were responsible for the greater part of the variation.

A preliminary analysis of the temperature effects on facet number was made by Seyster in this laboratory in 1916.

The white, bar-eyed mutant of *Drosophila* was used. Ordinary cultural procedure was followed, except that the banana was sterilized by bringing it to the boiling point. After cooling, the banana was inoculated with a pinch of Fleischmann's compressed yeast. 4 and 8 ounce wide-mouth bottles were used. After being fitted with one-fourth sheet of Scott Tissue Towelling and a tight cotton stopper, they were subjected to 120–150°C. in a Sargent dry air electric oven.

All matings were mass matings. Unselected stock refers to Stock 127, in which Zeleny started selection in 1917. Selected Low F_4 is a stock derived from Culture 150.3 of the fourth generation of these selections; Ultra-bar is a stock derived from a 19 facet male mutant in the F_2 low generation of the same selections. These parent stocks were maintained at 27°C. The low facet stocks were used more extensively because of the greater ease in counting, and also to eliminate as many germinal differences as possible (Zeleny).

Temperature Control.—A constant temperature of 15° was maintained in the cool room. The Johnson Heat Regulation System controls the temperature by forcing air over brine coils and redistributing it to the room. A constant temperature of 23° was maintained in a similar manner in the warm room.

A battery of aquaria, fitted with Johnson regulators controlling the flow of hot and cold water, supplied the following temperatures; —16, 17.5, 20, 25, and 30°C. 27° was maintained in a Chicago Surgical and Electric Company incubator. At first, very marked differences in temperature were found in various parts of this incubator (1.5–3°). A 9 inch desk fan was installed in the top of the incubator, with the blades inside and the motor outside. This fan was run at high speed, and no further temperature differences were noticed.

Temperature Records.—Temperature records were kept at 27° by a Tycos Recording Thermometer. At 15 and 23°, New Tycos Thermographs were used. In the aquaria, Friez Soil and Water Thermographs kept the records.

The experimental material was kept as close to the effective elements of these instruments as was possible. Checks were frequently made on the instruments by standardized thermometers.

The temperature of the banana in which the larvæ were developing was tested by inserting a thermometer through the cotton plug into the food. Only very slight differences were found between the food and the surrounding medium.

The temperatures as given in the experimental data vary at most ±0.5°C.

At the high temperatures, 29 and 31°, additional checks were made on the rate of metamorphosis and facet number by using a water thermostat. This instrument is of the type used by physical chemists. It is a battery jar supplied with a U-tube mercury regulator closing a secondary heating system by relay. A 3-inch, motor-driven fan keeps the water agitated. Variations in temperature do not exceed ±0.05°C.

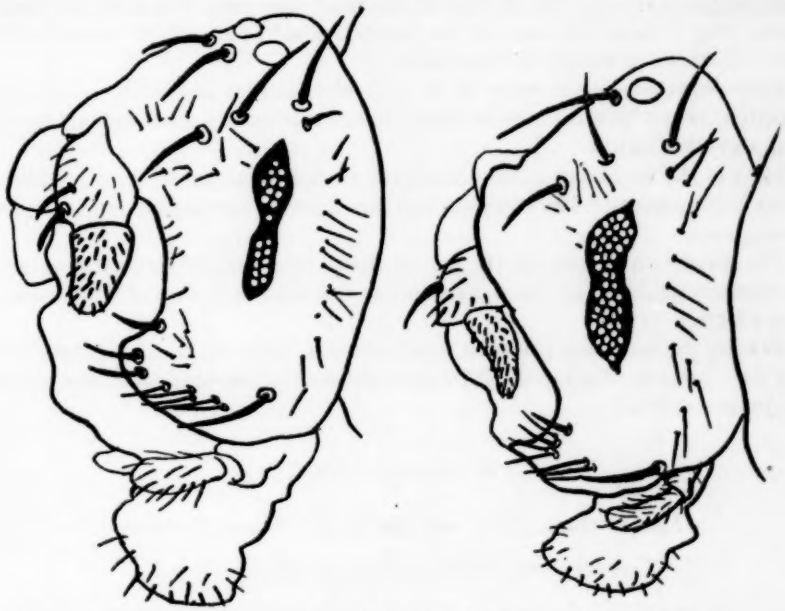

FIG. 1. Camera lucida drawings of the heads of two Ultra-bar females. The one on the left has 21 facets; reared at 27°. The one on the right has 48 facets, reared at 15°. The magnification is the same in both drawings. ×100.

Technique.—Counts of the facets were made by the methods described by May. The flies were etherized and placed one at a time on a wooden counting block. The upper surface of this block was cut to a slight incline in order to bring the compound eye into the best position for focusing. The facets were counted under a No. 4 ocular and a No. 3 Leitz objective with direct illumination from a 60 watt tungsten lamp. Adjustments were made with the fine thumb-screw to accommodate the curvature of the head. Facets were recorded immediately after each individual count. The material was then preserved in alcohol.

Sources of Error.—Errors due to technique have been discussed at length by May. The chief sources are (1) errors in counting, and (2) differences in counts between the right and left eyes. The latter factor is not significant in this study. Counts were made on one eye only, but no preference was given either eye in making the counts. The first factor was reduced to a minimum by using the low facet stocks. Ultra-bar at 15° has a mean of only 60.81 facets for the males. In such stock, all the facets are readily counted as no confusion of rows arises (Fig. 1).

There is no direct correlation between the size of the fly and the number of facets (Seyster, 1919). Exceptionally large individuals can be produced under the best cultural conditions at 31° and yet show the extreme low facet number of that temperature. All the flies are of practically the same size at all temperatures. Fig. 1 shows the heads of two females, raised at 27 and 15° respectively. They are drawn to exactly the same scale.

Slight variations in the mean of the individual bottles and between separate experiments may be attributed to slight differences in acidity, food, random sampling, and other factors.

Some of the variability in the Unselected stock may be due to germinal differences in the parents. The Ultra-bar and Low Selected bar stocks are practically homogeneous.

The precaution of keeping the parent stocks at a constant temperature was unnecessary as shown by the experiments on the non-inheritance of the temperature effects.

Bearing the foregoing points in mind, we may conclude that temperature is the chief factor involved in the differences obtained in the mean facet numbers in the present study.

EXPERIMENTAL.

Temperature Effect on the Mean Facet Number.

Temperature Effect on Unselected Bar Stock.

In 1916, Seyster carried out some experiments on the bar-eyed mutant of *Drosophila* showing that facet number varied inversely with the temperature at which the flies developed.

The preliminary experiments on Unselected bar stock confirm these results. Summaries of the first experiments are given in Tables I and II. They include the temperature at which the flies developed, the number of individuals whose facets were counted, the average or mean facet number for those individuals, the difference in the means accompanying a difference of 1°C. in the environment, and the per cent of change in the mean produced by a difference of 1°C.

TABLE I.

Unselected Bar Stock. Summary of Experiments 1, 4, 7, and 16. Females.

Temperature at which flies were reared.	No. of individuals counted.	Mean facet No.	Difference in facet No. for interval.	Difference in facet No. per °C.	Average of mean facet Nos. for interval.	Per cent of change in mean per °C.
°C.						
15	90	213.67				
			91.47	18.29	167.93	10.89
20	117	122.20				
			41.12	8.22	101.64	8.08
25	114	81.08				
			41.42	8.28	60.37	13.71
30	97	39.66				
			Average.	11.59		10.89

TABLE II.

Unselected Bar Stock. Summary of Experiments 1, 4, 7, and 16. Males.

Temperature at which flies were reared.	No. of individuals counted.	Mean facet No.	Difference in facet No. for interval.	Difference in facet No. per °C.	Average of mean facet Nos. for interval.	Per cent of change in mean per °C.
°C.						
15	71	269.76				
			109.00	21.80	215.26	10.12
20	109	160.76				
			40.24	8.05	140.64	5.72
25	109	120.52				
			46.98	9.39	97.03	9.68
30	92	73.54				
			Average.	13.08		8.50

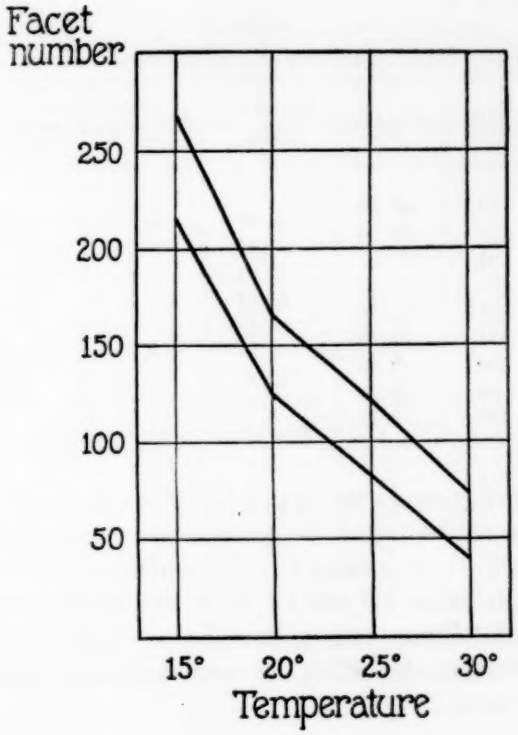

FIG. 2. Temperature effect on the mean facet number in Unselected bar stock. The upper curve is that of the males, the lower that of the females.

The difference in facet number per °C. is found by dividing the observed difference between the means of two temperatures by the number of degrees there are between those temperatures. The per cent change in the mean is found by dividing the difference in facet number per °C. by the average of the two means for the observed temperatures.

A consistent sexual difference exists in all these experiments. It is impractical to combine the data for the two sexes into a single table as the sex coefficient is not constant throughout.

The results of these experiments are shown graphically in Fig. 2.

Temperature Effect on Low Selected Bar Stock.

The preliminary experiments on Unselected bar stock showed the temperature range of the species to be practically limited to 15–30°C. From this it was decided to interpolate as many intermediate experi-

TABLE III.

Low Selected Bar Stock. Summary of Experiments 12, 22, and 27. Females.

Temperature at which flies developed.	No. of flies counted.	Mean facet No.	Difference in facet No. for interval.	Difference in facet No. per °C.	Average of means for interval.	Per cent of change for interval.
°C.						
15	79	189.00				
			30.80	30.80	173.59	17.74
16	104	158.20				
			30.93	20.61	142.73	14.43
17.5	94	127.27				
			28.39	11.36	113.07	10.04
20	105	98.88				
			24.63	4.93	86.56	5.69
25	165	74.25				
			19.12	9.56	64.69	14.73
27	164	55.13				
			7.73	3.86	51.26	7.54
29	100	47.40				
			10.84	10.84	41.89	25.82
30	64	36.56				
			7.71	7.71	32.70	23.57
31	100	28.85				
			Average.	12.38		14.95

mental temperatures as the apparatus at hand would permit. For the Low Selected bar stock the temperatures were as follows: 15, 16, 17.5, 20, 25, 27, 29, 30, and 31°C. Summaries of these experiments are included in Tables III and IV, while the results are shown graphically in Fig. 3. From these tables it is at once apparent that temperature has a very marked effect on the mean facet number of the Low Selected bar stock.

TABLE IV.

Low Selected Bar Stock. Summary of Experiments 12, 22, and 27. Males.

Temperature at which flies developed.	No. of flies counted.	Mean facet No.	Difference in facet No. for interval.	Difference in facet No. per °C.	Average of means for interval.	Per cent of change in mean per °C.
°C.						
15	74	240.21				
			32.66	32.66	223.88	14.58
16	107	207.55				
			42.89	28.59	186.10	15.36
17.5	95	164.66				
			41.78	16.71	143.77	11.62
20	104	122.88				
			19.76	3.95	113.00	3.49
25	164	103.12				
			23.66	11.83	91.29	12.95
27	167	79.46				
			13.88	6.94	72.52	9.56
29	100	65.58				
			7.78	7.78	61.69	12.61
30	61	57.80				
			16.10	16.10	49.75	32.36
31	100	41.70				
			Average.	15.57		14.06

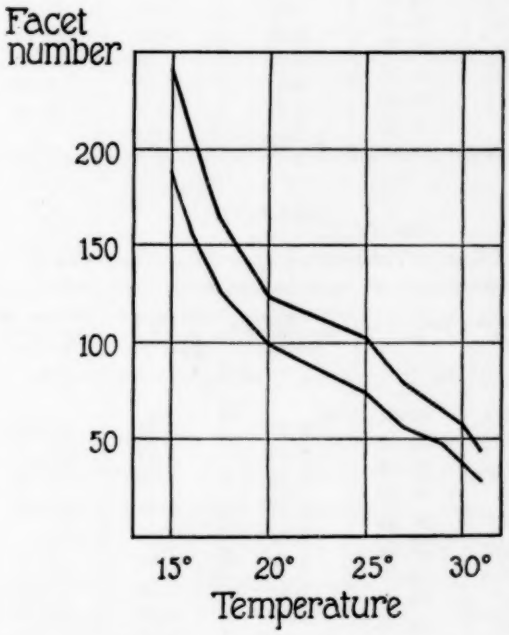

FIG. 3. Temperature effect on the facet number in Low Selected bar stock. The upper curve is that of the males, the lower that of the females.

Temperature Effect on Ultra-Bar Stock.

Experiments on this stock involve practically the same temperatures as the preceding ones. The summaries of the experiments on Ultra-bar stock are given in Tables V and VI and are shown in Fig. 4.

TABLE V.

Summary of the Effects of Temperature upon Facet Number in Ultra-Bar Females.

Temperature at which flies developed.	No. of flies counted.	Mean facet No.	Difference in facet No. for interval.	Difference in facet No. per °C.	Average of means for interval.	Per cent of change in mean per °C.
°C.						
15	55	51.51				
			8.24	8.24	47.39	17.37
16	101	43.27				
			4.70	3.13	40.92	7.64
17.5	112	38.57				
			5.98	2.39	35.58	6.72
20	112	32.59				
			4.29	1.43	30.49	4.69
23	94	28.30				
			3.06	1.53	26.77	5.72
25	500	25.24				
			3.97	1.98	23.25	8.51
27	490	21.27				
			4.04	2.02	19.25	10.49
29	629	17.23				
			2.66	1.33	15.90	8.36
31	138	14.57				
			Average.	2.76		8.58

TABLE VI.

Summary of the Effects of Temperature upon Facet Number in Ultra-Bar Males.

Temperature at which flies developed.	No. of flies counted.	Mean facet No.	Difference in facet No. for interval.	Difference in facet No. per °C.	Average of means for interval.	Per cent of change in mean per °C.
°C.						
15	65	60.81				
			9.71	9.71	55.95	17.35
16	103	51.10				
			5.92	3.95	48.14	8.21
17.5	100	45.18				
			7.98	3.19	41.19	7.74
20	120	37.20				
			5.77	1.92	34.31	5.59
23	94	31.43				
			3.83	1.91	29.51	6.47
25	469	27.60				
			3.90	1.95	25.65	7.60
27	538	23.70				
			4.68	2.34	21.36	10.95
29	641	19.02				
			4.86	2.43	16.59	14.65
31	187	14.16				
			Average.	3.42		9.82

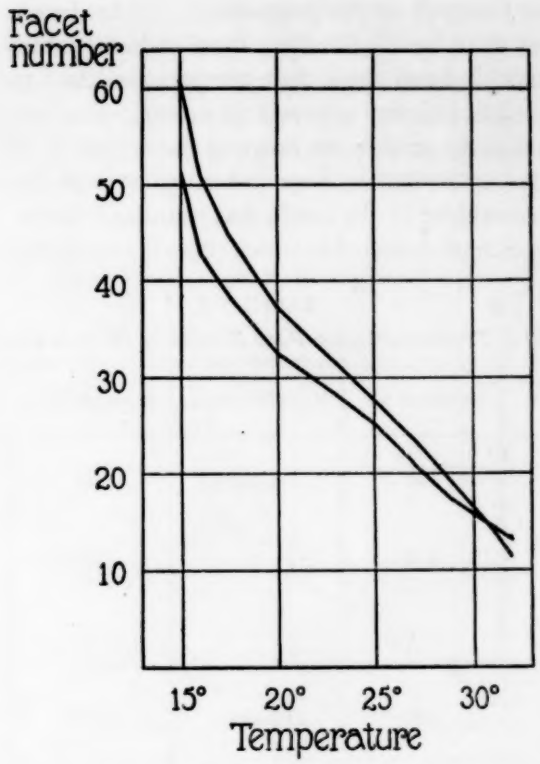

FIG. 4. Temperature effect on the mean facet number in the Ultra-bar stock. The upper curve is that of the males, the lower that of the females.

Temperature Effect on Full-Eyed Wild Stock.

In Table VII are given the counts of the full-eyed wild stocks reared at 15 and 27°.[2] They were made on material that was first boiled in caustic potash, cleared, and mounted in balsam. The slides were held in a mechanical stage while the facets were counted under a Zeiss No. 8 objective and a No. 12 ocular. The ocular field was broken up into rectangles by spider web cross hairs. One rectangle was counted at a time.

[2] The counts of the full-eyed wild stocks reared at 27° are available through the kindness of Dr. Zeleny.

The labor involved in the preparation and in the counting renders the full-eyed stock unsuitable for a more elaborate study.

The counts at hand show that temperature does not affect facet number in full-eye to any appreciable extent. One female of the two at 15° was slightly outside the range of the counts at 27°. The three flies mounted and counted were taken at random from a hatch at 15°. The remainder of the hatch was examined under the binocular. No differences were detected between these flies and the three counted

TABLE VII.

Effect of Temperature upon Facet Number in the Full-Eyed Stock.

Temperature at which flies were raised.	Experiment No.	No. of individuals.	Average facet No.	Range in facet No.
°C.				
27	398	5 females	956	886 1,082
27	345	10 "	810.6	632 924
15	51.3	2 "	1,084	912 1,256
27	345	10 males.	849.8	700 980
15	51.3	1 male.	1,016	

Temperature Effects on the Three Bar Stocks Compared.

For a direct comparison, the mean facet numbers of the females of the three stocks are shown in Fig. 5. Here they are all plotted to the same scale. The lower curve is that of the Ultra-bar stock, the middle one is that of the Low Selected bar stock, while the upper one is that of the Unselected bar stock.

In the Ultra-bar stock, the mean for the females is reduced from 51.51 facets at 15° to 21.27 at 27°. In the Low Selected, the mean is reduced from 189.00 facets at 15° to 55.13 at 27°. In the Unselected bar stock, the mean is 213.67 at 15° and 81.08 at 25°. There is a very marked reduction in facet number with increase in temperature in each of these three stocks.

The Low Selected and the Unselected bar stocks parallel each other closely in their temperature-facet relations. The Low Selected has a consistently lower mean facet value for all temperatures studied.

The Ultra-bar stock differs from the Low Selected first in the mean facet values for all temperatures, and, second, in the amount of reduction for a degree Centigrade change in temperature.

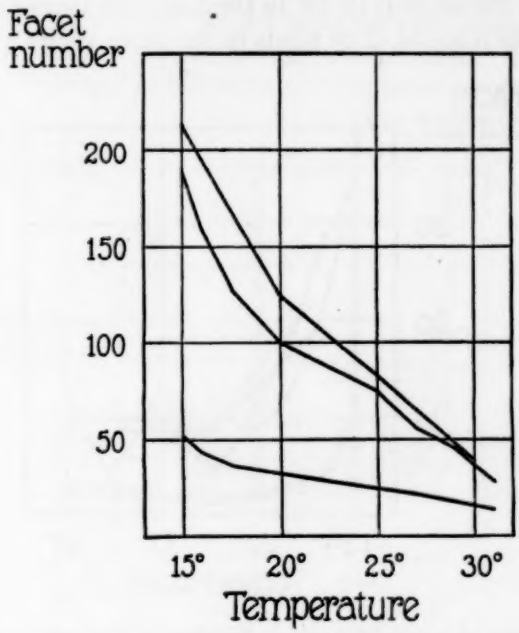

FIG. 5. Temperature effect on the three bar stocks compared. The three curves are all drawn to the same scale. The upper one is that of the Unselected bar stock females, the middle that of the Low Selected bar stock females, and the lower that of the Ultra-bar stock females.

We may draw two conclusions from these curves: (1) The mean facet number at any given temperature is not the same for all stocks. (2) The difference in the mean number of facets between any two temperatures is not a constant for all three stocks. In other words, the number of facets is determined by a specific germinal constitution plus a specific environment.

The Temperature Coefficient.

(*a*) *Changes in Mean Facet Number Accompanying a Unit Temperature Change.*—In the fifth rows of Tables I to VI are given the average differences in facet number per °C. for the temperature intervals indicated. These temperature differences are shown in Fig. 6.

The greatest change in facet number for a change in temperature of 1° is that for the interval 15–16° in the Low Selected males. Here 1° produces a difference of 32.66 facets in the mean values.

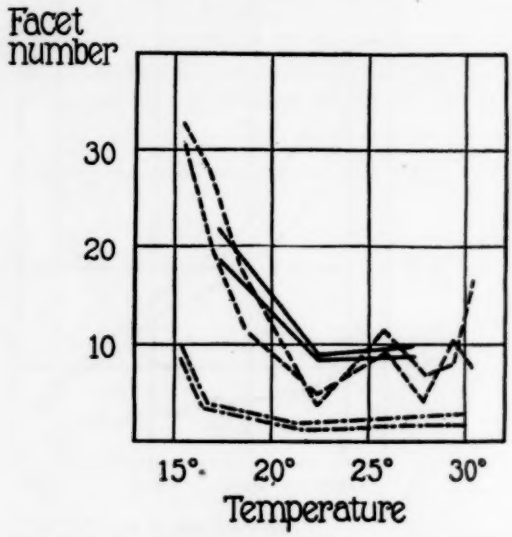

FIG. 6. Number of facets difference in the mean accompanying a difference of 1° in temperature in the environment in which the flies were raised. The solid line represents the Unselected bar stock, the broken line the Low Selected, and the dot and dash line the Ultra-bar. The upper curve of a pair is for the males in each case.

The least change for a difference of 1° in temperature is for the interval 29–31° in the Ultra-bar females. Here a difference of 1° in temperature produces only 1.33 facets difference between the two means.

Obviously a difference of 1° does not produce a constant difference in the mean number of facets. The greater differences are produced at the lower temperatures, the lesser at the higher. Again, the greater

differences are produced in the high mean stocks while the lesser are produced in the low mean stocks.

The average difference in facet number accompanying a difference of 1°C. in the environment in which the flies were reared is 3.09 for the Ultra-bar and 14.01 for the Low Selected bar stock.

(*b*) *Per Cent of Change in the Mean Facet Number Accompanying a Unit Change in Temperature.*—In the lower rows of Tables I to VI, the change per °C. is expressed in per cents of the mean. These values are found by dividing the difference in facet number per °C. for the

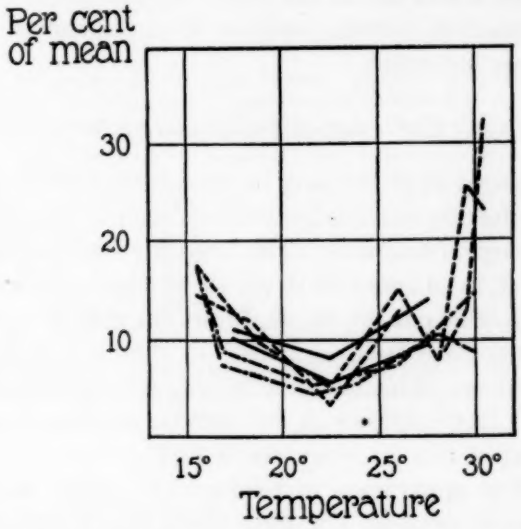

FIG. 7. Per cent of change in mean facet number accompanying a change of 1° in temperature. The solid line represents the Unselected bar stock, the broken line the Low Selected, and the dot and dash line the Ultra-bar. The upper curve in each pair represents the males.

interval by the average of the mean values for the two temperatures.

The per cent of change per °C. in Ultra-bar varies from 17.39 to 4.69; in Low Selected, from 32.45 to 3.49; in Unselected, from 13.71 to 5.84. Thus the proportional change per °C. is (1) not constant for all temperatures or (2) constant for the three stocks.

The average per cent change per °C. in the mean of the Ultra-bar is 9.22; for Low Selected it is 14.01.

(*c*) *Relative Change at the Different Temperatures.*—All three stocks show one feature in common. The extreme high (29–31°) and low (15–17.5°) temperatures produce the greatest changes in facet number per °C. when the differences are expressed in per cent of the mean. The lowest proportional changes are produced by the intermediate temperature at or near 23° (Fig. 7).

Except for this phenomenon of increased change at the extreme temperatures, the difference in facet number accompanying a change of 1°C. is roughly proportional (about 10 per cent) to the mean facet number. Thus a decrease in the mean, whether produced by germinal or environmental factors, produces a corresponding decrease in the temperature increment.

Application of van't Hoff's Law to Temperature Effect on Facet Number.

Many investigators in the past 10 years have attempted to obtain an expression for the relationship between temperature and the rates of various biological reactions. The interest in the problem has been twofold. First, in an endeavor to reduce all vital processes into terms of dynamics, sorting out the physical from the chemical, the temperature coefficients have been compared to those of various physicochemical reactions. This has been mostly through the direct application of van't Hoff's law, which for chemical reactions means a doubling or trebling of rate for every rise of 10°C.

The practical application of temperature effects has developed another group of workers. For them van't Hoff's formula was very unsatisfactory. They give a physiological interpretation and represent the rate of vital processes as a linear function of the temperature.

The formula of van't Hoff and the physiological formula as given by Krogh are as follows:

$$v_{t+1^\circ} = v_t \times Q_1; \qquad v_{t+10^\circ} = v_t \times Q_1^{10} = v_t \times Q_{10}$$
$$v_{t+1^\circ} = v_t + K_1; \qquad v_{t+10^\circ} = v_t + 10K_1 = v_t + K_{10}$$

where v is the velocity of the process, t is the temperature in °C., Q_1 and Q_{10} are the van't Hoff constants, and K_1 and K_{10} are the Krogh constants.

The Q_{10} values, when calculated directly for intervals of 10°, closely approximate the theoretical demands of van't Hoff's law (Tables VIII and IX).

When the Q_{10} values are calculated from the Q_1, Q_2, etc., an interesting fact is discovered. In Tables X and XI, I have calculated the Q_{10} values from the formula $Q_{10} = (Q_{t_1-t_2})^{\frac{10}{t_1-t_2}}$. The values of Q_{10} vary from 5.69 to 1.60. The lower values are at the median temperatures while the higher ones are at the extremes. This is not the characteristic variation in Q_{10} for chemical reactions. The values consistently decrease in the latter with increase in temperature.

TABLE VIII.

Q_{10} Calculated Directly for Ten Degree Interval. Ultra-Bar Females.

Temperature interval.	Facet No.		Q_{10}
°C.			
15–25	51.51	25.24	2.04
17–27	40.14	21.27	1.89
20–30	32.59	15.90	2.03

TABLE IX.

Q_{10} Calculated Directly for Ten Degree Interval. Low Selected Bar Females.

Temperature interval.	Facet No.		Q_{10}
°C.			
15–25	189.00	74.25	2.54
20–30	98.88	36.56	2.79

In Fig. 8 the curve for the facet numbers in the Ultra-bar females is superimposed on a theoretical van't Hoff curve. In the latter the rate at 10°C. is arbitrarily taken as 10; from which at 20°C. it becomes 20 and at 30°C. it is 40. Intermediate values are interpolated. The experimental curve is fitted to the other by taking the value of "facets" at 20°C. as 20 and then applying the $Q_{t_1-t_2}$ as given in Table X. It is plainly evident that the experimental curve for the effect of temperature upon facet number is something more than a chance approximation of the theoretical van't Hoff curve.

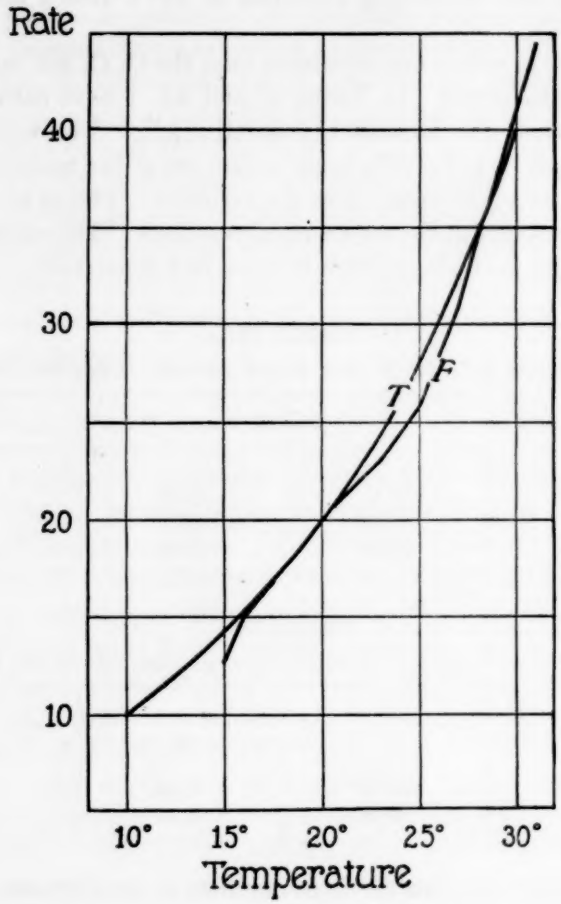

FIG. 8. A theoretical van't Hoff curve (T). The rate at 10° is arbitrarily taken as 10, at 20° it becomes 20, and at 30° it is 40. The reciprocal of the Ultra-bar female facet number curve (F) is shown superimposed. The curves are thrown into juxtaposition by taking the value 20 for facets at 20° and then calculating the remaining values by applying the $Q_{t_1-t_2}$ values, as given in Table X.

TABLE X.

Q_{10} (Calculated from $Q_{t_1-t_2}$) for Facet Number in Ultra-Bar Females.

Temperature.	Mean facet No.	$Q_{t_1-t_2}=\frac{M_1}{M_2}$	Q_{10}
°C.			
15	51.51		
		1.19	5.69
16	43.27		
		1.12	2.15
17.5	38.57		
		1.18	1.96
20	32.59		
		1.15	1.60
23	28.30		
		1.12	1.77
25	25.24		
		1.18	2.36
27	21.27		
		1.23	2.86
29	17.23		
		1.18	2.33
31	14.57		

TABLE XI.

Q_{10} (Calculated from $Q_{t_1-t_2}$) for Facet Number in Low Selected Females.

Temperature.	Mean facet No.	$Q_{t_1-t_2}=\frac{M_1}{M_2}$	Q_{10}
°C.			
15	189.00		
		1.19	5.92
16	158.20		
		1.24	4.26
17.5	127.27		
		1.28	2.74
20	98.88		
		1.33	1.77
25	74.25		
		1.34	4.43
27	55.13		
		1.16	2.12
29	47.40		
		1.29	13.36
30	36.56		
		1.26	10.66
31	28.85		

Temperature Effect on the Rate of Development.

The time-temperature curves, representing the number of days necessary to pass through a given stage in the life history, give an interesting set of data with which to compare the facet curves. Table XII gives the number of days at the respective temperatures necessary to complete the egg, larval, and pupal stages. Loeb has treated in full the subject of the length of the various metamorphic phases in *Drosophila* as affected by temperature. As my results are consistent with his in as far as the type of curve is concerned, I shall say nothing

further on this point. My values for the length of the egg-larval-pupal period do not coincide in some particulars with his, due to a difference in technique. My flies were subjected to the experimental temperatures immediately upon mating.

With my cultural methods, I was unable to get the Ultra-barstock to develop beyond pupation at 33°C. The data given in the developmental curve for this temperature are for wild full-eyed stock. There is some variation in the length of the periods as shown by the individual cultures. All sets made up at the same time and with the same food, however, give the type of curve shown by the averages (Table XII).

TABLE XII.

Effect of Temperature upon the Length of the Immature Period (Egg-Larval-Pupal) in Drosophila.

Temperature.	Mean length in days of immature period.	Reciprocal or rate per day.
°C.		
15	31.87	0.0313
16	22.93	0.0436
17.5	19.20	0.0521
20	13.62	0.0734
23	11.75	0.0851
25	10.38	0.0964
27	9.22	0.1084
29	8.27	0.1209
30	9.03	0.1107
31	9.21	0.1085
33	9.42	0.1062

In Fig. 9 is shown the number of days from mating of the parents to the emergence of the offspring from pupation. The reciprocal, or rate per day, curve is also given. This rate curve gives approximately a straight line between 15 and 29°C. Beyond 29° it turns down, thus showing an optimum.

This type of temperature curve is found for nearly all physiological reactions. Krogh has recalculated Loeb's data and has shown that they represent the time-temperature relations for the rate of segmentation of the sea urchin eggs far better than the van't Hoff formula. In the experimental data of various workers he has demonstrated this

type of curve. He has demonstrated the straight line curve in the rate of CO_2 production by *Tenebrio* larvæ. Sanderson, Headlee, Peairs, and many others have shown that all the various phases of insect metabolism investigated follow this principle. Lehenbauer, as the

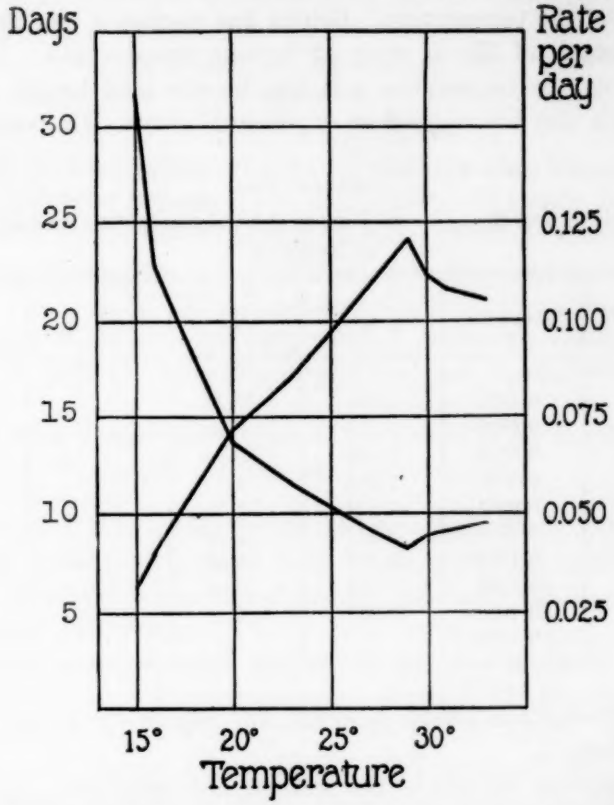

FIG. 9. Temperature effect on the length of the immature stages (egg, larval, and pupal) and on the rate of development. The curve that starts at the top to the left represents the number of days from the mating of the parents to the emergence from pupation of the offspring. The reciprocal represents the percentage of total development completed in 1 day.

most recent worker on rate of plant growth, has shown the same relations for maize seedlings. An examination of many curves dealing with the effect of temperature on the rate of growth and the degree

of infection of parasitic fungi demonstrates the same principle here. Simpson and Rasmussen's data for the rate of coagulation of blood give a similar curve.

Snyder, Fallas, and Elmendorf, dealing with the rate of heart beat of the cat, maintain that this reaction is a logarithmic and not a linear function of the temperature. Groves has applied a similar formula to the length of life of seeds at various temperatures. Loeb has worked out the temperature relations for the total length of life of *Drosophila* and has applied an exponential curve. He compromises

TABLE XIII.

Q_{10} (Calculated from $Q_{t_2-t_1}$) for the Velocity of Development of the Immaturity Stages of Drosophila.

Temperature.	Rate per day.	$Q_{t_2-t_1}=\frac{V_2}{V_1}$	Q_{10}	$Q_{t_1-t_2}=\frac{V_1}{V_2}$	Q_{10}
°C.					
15	0.0313	1.392	27.31		
16	0.0436	1.195	3.28		
17.5	0.0521	1.409	3.94		
20	0.0734	1.313	1.72		
25	0.0964	1.124	1.79		
27	0.1084	1.115	1.72		
29	0.1209	0.915	0.41	−1.092	−2.41
30	0.1107	0.980	0.82	−1.020	−1.22
31	0.1085	0.978	0.89	−1.021	−1.11
33	0.1062				

on the larval-pupal period, however, and admits the straight line relations there.

An important feature of the straight line temperature-rate curve is that it holds only between certain temperatures. As already pointed out for the high temperatures, the rate decreases with increase in temperature above the optimum. Another feature of the straight line curve is that at the lower temperatures the rates are higher than they theoretically should be. The first feature is noted in these experiments; the second was not, as the lowest experimental temperature was not the minimum for development. In Table XIII are shown the Q_{10} values for the rate of development as calculated from

the $Q_{t_2-t_1}$ experimental values. For the 16–15° interval, $Q_{10} = 27.31$; for the 17.5–16° interval it is 3.28; there is a decrease in the value from here as the temperature increases until at 33–31° the value is 0.89.

I wish to point out here that above 29° the values of Q_{10} really become negative.

At 29 – 30° $Q_{10} = -2.41$
" 30 – 31° $Q_{10} = -1.22$
" 31 – 33° $Q_{10} = -1.11$

Obviously Q_{10} has no practical value for these reactions since its value may lie anywhere between 27.31 and −2.41. A glance at Lehenbauer's data on the rate of growth of maize seedlings shows even a greater departure from the theoretical 2–3 value of Q_{10}. Here it ranges from 32 at 15–13° to −428.2 at 42–45°C. Exceedingly high temperature coefficients are suggestive of enzymatic reactions and toxicity effects while the negative values suggest physical processes.

Relation of the Temperature Effects on the Length of the Developmental Periods to Those on Facet Number.

We have seen that the facet curve can be superimposed on the theoretical Q_{10} curve with a closeness that can hardly be attributed to chance. The next obvious thing is to see how closely the facet and the metamorphic curves agree.

In Fig. 10, the facet curves for the Low Selected bar stocks are superimposed on the development curve. From 15–25° the two sets of curves approximate each other very closely. Above 25°, the metamorphic curve decreases less rapidly, until above 29° it begins to turn up again. The facet curves continue to decrease at their initial rate.

The reciprocal curves are of particular interest. That for the rate of development gives approximately a straight line between 15 and 29°. It likewise shows an optimum at 29° with a subsequent decrease in rate and further increase in temperature. The reciprocal of the facet curve gives an exponential curve, without a decrease in rate at the upper temperatures. The lower part of this curve approximates the straight line features of the metamorphic curve. In other words,

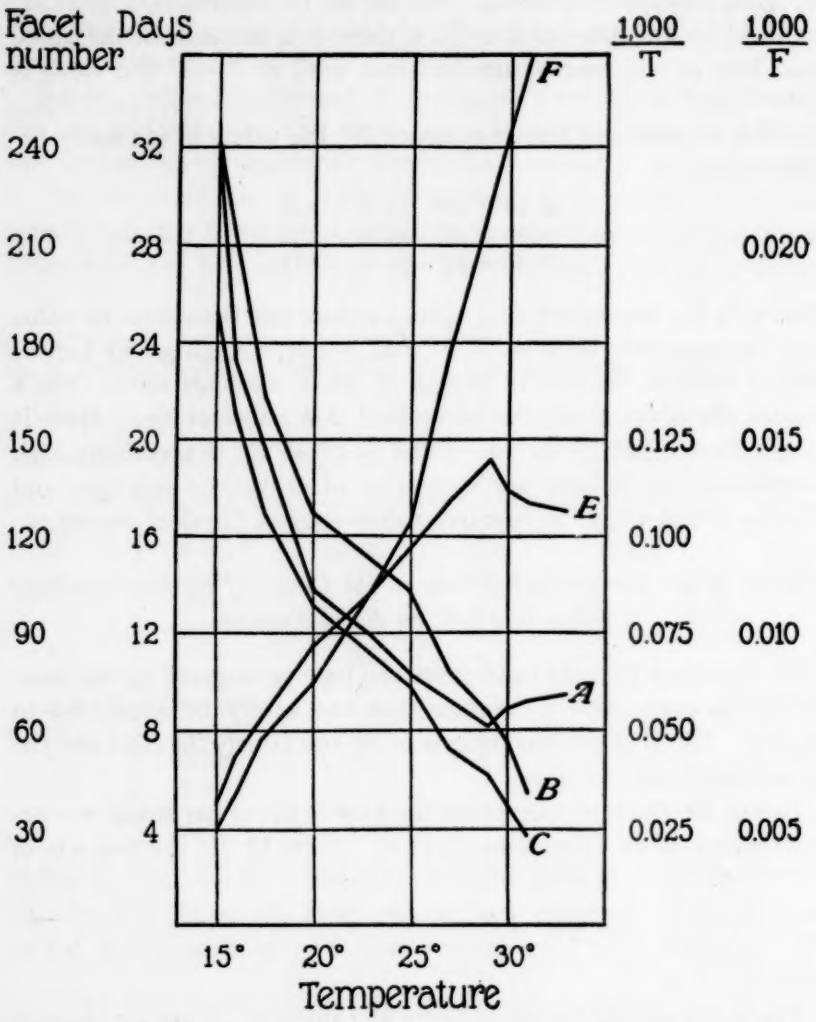

FIG. 10. The Low Selected bar stock facet curves superimposed on the curve for the length of development of the immature period. These curves are thrown into juxtaposition by arranging the values so that the developmental curve A lies about half way between the male facet curve B and the female facet curve C. The reciprocal curve E for the rate of development and the reciprocal female facet number curve F are likewise brought into comparison by arranging the values for each.

the length of the developmental period and the number of facets are correlated up to the point where the time-temperature relations for metamorphosis begin to fail.

This leads to the question; Is the number of facets dependent upon the length of the immature stage?

TABLE XIV.

Experiment 6. Unselected Bar Stock. Larvæ Subjected to 30° for First Part of Development; Remainder at 15°.

No. of days at 30° before removal to 15°.	1		2		3		4		5		6		7	
Facet No. classes.	Distribution of individual counts.													
15–44							4	1	7	3	4	2	10	3
45–74							4	7		10			2	7
75–104							6	2	4	2	1			
105–134							5	1	3	2				
135–164					2		1	1	2	1				
165–194					3	4	5	1	3	5				
195–224	3	1	7	1	10	4	6	3						
225–254	6	3		2	11	6		2						
255–284	1	1	1	3	2	6	2	1						
285–314		4			1	4		4						
315–344		1				2								
Mean facet No.	230	279	216	251	221	246	152	224	93	95	44	59	36	48
Total No. of days to complete immature stages	25		24		23		22		22		20		18	

Control stocks.				
At 15° throughout.			At 30° throughout.	
Facet No.	213	269	39	73
Total No. of days in period	31.87		9.02	

In the previous experiments it is obvious that there is a certain degree of correlation between the facet number and the length of the egg-larval-pupal period. Are these two phenomena directly dependent or are they separately affected by a third common factor? Experiment 6 was designed for another purpose but it supplies data here.

In Table XIV is given the distribution of facet counts in Experiment 6 in which the successive bottles, made up by changing the same par-

ents daily, were treated to varying periods at 30° before subsequent development at 15°. The upper row in the table gives the number of days at 30°. Below the class distributions are given the mean facet values for males and females. In the lower row is given the number of days required to complete the immature stage.

It is evident that there is no direct causal relation between the number of days taken to complete the immature stage and the number of facets. 25, 24, and 23 days give practically the same facet counts that are obtained when total development is passed at 15°. In the last case 31 days are required to complete the immature stages. Again, those flies hatching at 18 and 20 days are in the same facet classes as those hatching in the stock experiments in 9 days at 30°.

Why, then, is there an apparent correlation between the facet count and the length of the immature period when the latter is passed throughout at one temperature? We shall return to this question at the end of the succeeding part of this study.[3]

The author wishes to express gratitude to Dr. Charles Zeleny, under whose direction the work was done.

Acknowledgments are also due to Doctor V. E. Shelford for many valuable suggestions and to Doctor G. Dietrichson for the loan of apparatus.

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